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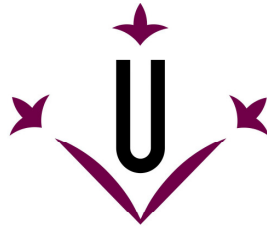
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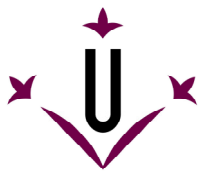
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To my daughter Alisa

Contents

Summary

In dryland fields in NE Spain, the harvester ant *M. barbarus* is the main seed predator. Post-dispersal seed predation by harvester ants is high (78%) in spring and summer. One characteristic of seed predation is that it is variable over time and space. This variability can influence the efficacy of granivory and may provide opportunities to weeds to escape predation. However, factors that determine the temporal and spatial variability in predation rates are largely unknown. This knowledge could help to better understand the constraints and potentials of seed predation by harvester ants for weed management.

Seed predation rates by harvester ants in dryland cereals were among the highest ever recorded on arable fields (46-100%). The reason for the high rates is that the timing of weed seed shed overlapped with the period of highest demand. However, a small proportion of newly shed seeds were not consumed because of crop harvest or low preference. Some seeds escaped predation due the small size that allowed fast seed burial. Of the seeds of *Bromus diandrus* about 15 to 25% avoided predation because of low preference to ants, while 0–29% escaped through crop harvest. Of the seeds of *Papaver rhoeas,* 20–32% escaped through crop harvest, while another 13–17% escaped by fast burial into the soil. It is clear that both would cause more problems in arable fields without the presence of seed predators.

The spatial distribution of ant nests was investigated because it could influence seed predation rates due to unequal foraging intensities within a field. The spatial nest arrangement appeared to be non-random. The large nests were more or less regularly distributed, but small nests tended to be more clumped. The clumped distribution of small nests can be understood by the process of new queen establishment and competition with

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larger nests. No reason was found to explain the nest density decrease from 2009 to 2010. The clumped distribution trend decreased with increasing density of colonies; however it was observed an over-dispersion trend of small nests in 2009. The regular trends in nest distribution increased with nest density and decreased opportunity to weed seeds to escape predation due the higher predation rates in the field.

The effect of patch quality, defined as the seed density in a patch, on seed predation by harvester ants was investigated. Seed predation in patches is composed of two components, namely the patch encounter rate and the patch exploitation rate. The encounter rate was independent of the seed density in a patch. Harvester ants responded with extremely high predation rates (99-100%) to all densities of *Lolium multiflorum* seeds applied (1000-20000 seeds m^{-2}), and the response was density independent. The most likely explanation is that nest density in the field was so high (>300 nests ha^{-1}). The few patches that were not found occurred in areas with a low nest density.

Patch size influenced predation rates by harvester ants. Estimated seed predation rate was highest in the largest patches (99-100%), and lowest in the smallest patches (78-94%). This was caused by a lower encounter rate of small patches. Larger patch size facilitated patch encounter rate due to larger perimeters and higher area-to-perimeter ratios (A/P). When patches were found, the exploitation rate was the same, regardless of the size.

The current research quantified temporal and spatial variability in seed predation in dryland cereals to investigate how this variability might influence the efficacy of granivory and provide opportunities to weeds to escape predation. In order to decrease or remove constraints for this natural service, measures to improve conditions for nest

establishments in areas with a low presence of harvester ants could be considered. If farmers want to make optimal use of seed predation, they could decide to delay harvest to allow more seeds to be shed and predated by ants. Alternatively, farmers could remove the straw immediately after harvest to make seeds better accessible. It was confirmed that the spatial distribution of harvester ant nest densities were not constant within a field, so that areas remained with lower nests, and lower predation rates should be expected. Adopting measures to preserve ant nests in areas with high densities or increase them in areas with lower abundances should be further researched and considered by farmers.

Resumen

En los cereales de secano del noreste de España, la hormiga granívora *Messor barbarus* es el principal depredador de semillas de malas hierbas. La depredación después de la dispersión de las semillas es alta en primavera y verano (78%). Aun así, la depredación de semillas es variable tanto en el espacio como en el tiempo. Esta variabilidad puede influenciar la eficacia de la granivoría y dar oportunidades a las malas hierbas para escapar de ella. Por el contrario, los factores que determinan esta variabilidad espacio-temporal en los niveles de depredación son mayormente desconocidos. Este conocimiento podría ayudar a entender mejor las limitaciones y potencialidades de la depredación de semillas por hormigas granívoras para el manejo integrado de malas hierbas.

Los niveles de depredación por hormigas granívoras en cereales de secano de este estudio fueron de los más altos jamás registrados en cultivos extensivos (46-100%). La razón fue la coincidencia en el tiempo de la producción de semillas de las malas hierbas con el período de máxima actividad de las hormigas. En contraste, una pequeña proporción de las semillas producidas no fue consumida debido a la cosecha y/o baja palatabilidad para las hormigas. Además, algunas semillas no fueron consumidas porque eran muy pequeñas y se enterraron rápidamente en el suelo. De un 15 a 25% de las semillas de *Bromus diandrus* no fueron consumidas porque fueron poco atractivas para las hormigas, mientras que un 0-29% no fueron depredadas debido a la cosecha. Un 20-32% de las semillas de *Papaver rhoeas* escaparon a través de la cosecha, mientras que un 13–17% porque rápidamente se enterraron en el suelo debido a su minúsculo tamaño. Es claro que las citadas especies, podrían causar problemas mucho más graves en estos

campos cerealistas sin la regulación población que de forma natural ejercen las hormigas al comer sus semillas.

La distribución espacial de los nidos de hormigas fue investigada porque podría afectar los niveles de depredación de semillas que podrían ser desiguales dentro de un mismo campo. El patrón de distribución de los nidos era no aleatorio. Los nidos grandes estaban distribuidos de forma más regular, mientras que los nidos pequeños estaban más agregados. La distribución agregada de los nidos pequeños se pueden entender por la baja supervivencia de las nuevas reinas fundadoras de colonias cerca de los nidos grandes y la competencia entre ellos. No hay una explicación clara de la disminución de nidos de 2009 a 2010. La distribución agregada de los nidos pequeños tendió a disminuir con densidades crecientes, aunque se observó una sobre dispersión de éstos en 2009. La distribución regular de los nidos tendió a incrementar al aumentar las densidades y disminuyó las posibilidades de las semillas de malas hierbas de escapar a los altos niveles de depredación en el campo.

La posible influencia de la calidad de los rodales de semillas de malas hierbas, en concreto la densidad de semillas, en los niveles de depredación por hormigas granívoras fue estudiado. El proceso de depredación de semillas está compuesto de dos componentes, la tasa de encuentro y la tasa de explotación del rodal. La tasa de encuentro fue independiente de la densidad. Las hormigas respondieron con altísimos niveles de depredación (99-100 %) a todas las densidades de semillas de *Lolium multiflorum* aplicadas (1000-20000 semillas m^{-2}), y la respuesta fue denso-independiente. La explicación más plausible es que la densidad de nidos en el campo era lo suficientemente

alta (>300 nidos ha^{-1}). Sólo algunos rodales no fueron encontrados en zonas con baja densidad de nidos.

El tamaño del rodal también influyó en los niveles de depredación de las hormigas granívoras. Estos fueron máximos para los rodales más grandes (99-100%), y más bajos para los rodales más pequeños (78-94%). Esto fue debido a la menor tasa de encuentro de los rodales más pequeños. Los rodales más grandes fueron más fácilmente encontrados debido a un perímetro más largo y una mayor relación área/perímetro. Cuando un rodal era encontrado, los niveles de explotación eran máximos independientemente de su tamaño.

La presente investigación cuantificó la variabilidad espacio-temporal de la depredación de semillas por hormigas granívoras en los cereales de secano para comprender como esta variabilidad afecta la eficiencia del proceso en el control de las poblaciones de malas hierbas y éstas pueden perdurar en los cultivos. Para eliminar o disminuir las limitaciones de este servicio ecológico del agro-ecosistema, habría que considerar medidas para favorecer las condiciones que permitieran un mejor establecimiento de colonias de hormigas en las zonas del campo con baja presencia. Si los agricultores quieren optimizar este servicio ecológico, podrían retrasar la fecha de cosecha para aumentar el tiempo de coincidencia de la producción de semillas de las malas hierbas con el período de máxima actividad de las hormigas. Alternativamente, también podrían retirar la paja inmediatamente después de la cosecha para reducir lo máximo posible el tiempo que las semillas están cubiertas y por tanto, inaccesibles a las hormigas. Se confirmó que las densidades de nidos de hormigas no son altas y constantes en todo el campo, y que había áreas con menos abundancia de nidos, y por lo tanto en

esas zonas se podrían esperar menores niveles de depredación. Posibles medidas para preservar los nidos en zonas de alta densidad o aumentarlos en áreas con baja abundancia deberían ser investigadas y consideradas por los agricultores.

Resum

La formiga granívora *Messor barbarus* es el principal depredador de llavors de males herbes en els cereals de secà del nord-est d'Espanya. La depredació després de la dispersió de llavors és alta a la primavera i estiu (78%). Tot i així, la depredació de llavors és variable tant en l'espai com en el temps. Aquesta variabilitat pot influenciar la eficiència del granivorisme i donar oportunitats a les males herbes per escapar-ne. Per altra banda, els factors que determinen aquesta variabilitat espacio-temporal en els nivells de depredació son bàsicament desconeguts. Aquest coneixement podria ajudar a entendre millor les limitacions i potencialitats de la depredació de llavors per formigues granívores per al maneig integrat de males herbes.

Els nivells de depredació de formigues granívores en cereals de secà d'aquest estudi van ser dels més alts mai registrats en cultius extensius (46-100%). La raó va ser la coincidència en el temps de la producció de llavors de males herbes amb el període de màxima activitat de les formigues. En contrast, una petita proporció de llavors produïdes no van ser consumida degut a la collita i/o baixa palatabilitat per a les formigues. A més, algunes llavors no van ser consumides perquè eren molt petites i s'enterraven amb facilitat en el sòl. D'un 15 a 25% de les llavors de *Bromus diandrus* no van ser consumides perquè eren poc atractives per a les formigues, mentre que un 0-29% no van ser depredades degut a la collita. Un 20-32% de les llavors de *Papaver rhoeas* van escapar a través de la collita, mentre que un 13–17% perquè es van enterrar ràpidament en el sòl. En qualsevol cas, és clar que les citades espècies, podrien causar problemes molt més greus en aquests camps de cereals sense la regulació poblacional que de forma natural fan les formigues al menjar les seves llavors.

La distribució espacial dels nius de formigues va ser investigada perquè podria afectar els nivells de depredació de llavors que podrien ser desiguales dins de un mateix camp. El patró de distribució dels nius era no aleatori. Els nius grans estaven distribuïts de forma més regular, mentre que els nius petits estaven més agregats. La distribució agregada dels nius petits se podria entendre per la baixa supervivència de les noves reines fundadores de colònies prop de nius grans i la competència entre ells. No hi ha una explicació clara de la disminució de nius de 2009 a 2010. La distribució agregada dels nius petits va tendir a disminuir amb densitats creixents, tot i que es va observar una sobre dispersió d'aquestos el 2009. La distribució regular dels nius va tendir a incrementar a l'augmentar les densitats i va disminuir les possibilitats de les llavors de males herbes d'escapar als alts nivells de depredació en el camp.

La possible influència de la qualitat dels rodals de llavors de males herbes, en concret la densitat de llavors, en els nivells de depredació de formigues granívores va ser estudiat. El procés de depredació de llavors està compost de dos components, la taxa d'encontre i la taxa d'explotació del rodal. La taxa d'encontre va ser independent de la densitat. Les formigues van respondre amb altíssims nivells de depredació (99-100%) a totes les densitats de llavors de *Lolium multiflorum* aplicades (1000-20000 llavors m^{-2}), i la resposta va ser denso-independent. L'explicació més plausible és que la densitat de nius en el campo era prou alta (>300 nius ha^{-1}). Només alguns rodals no van ser trobats en zones de baixa densitat de nius.

La mida del rodal també va influir en els nivells de depredació de les formigues granívores. Aquests van ser màxims per als rodals més grans (99-100%), i inferiors per als rodals més petits (78-94%). Això fou degut a la menor taxa d'encontre dels rodals més

petits. Els rodals més grans van ser més fàcilment trobats perquè tenen un perímetre més llarg i una major relació àrea/perímetre. Quan un rodal va ser trobat, els nivells d'explotació van ser màxims independentment de la mida.

Es va quantificar la variabilitat espacio-temporal de la depredació de llavors de formigues granívores en cereals de secà per comprendre com aquesta variabilitat afecta l'eficiència del procés en el control de poblacions de males herbes i aquestes perduren en els cultius. Per eliminar o disminuir les limitacions d'aquest servei ecològic del agro-ecosistema, s'haurien de considerar mesures per afavorir les condicions que permetin un millor establiment de colònies de formigues en les zones del camp amb baixa presència. Si els agricultors volen optimitzar aquest servei, podrien retardar la data de collita per augmentar el temps de coincidència de la producció de llavors de males herbes amb el període de màxima activitat de les formigues. Alternativament, també es podria retirar la palla immediatament després de la collita per reduir el màxim possible el temps que les llavors estan cobertes i per tant, inaccessibles a les formigues. Es va confirmà que les densitats de nius de formigues no són altes i constants en tot el camp, i que havia àrees amb menor abundància de nius, i per tant en aquestes zones se podrien esperar menors nivells de depredació. Possibles mesures per preservar els nius en zones d'alta densitat o augmentar-los en àrees amb baixa abundància haurien de ser investigades i considerades pels agricultors.

CHAPTER 1

General introduction

General introduction

Weed control as the main cost in agriculture

At a global level, weeds can cause losses of up to US $95 billion in agriculture, much higher than losses caused by pathogens ($85 billion), insects ($46 billion) or vertebrates excluding humans ($2.4 billion) (FAO, 2012). Despite the high costs, in most countries farmers continue to use herbicides because of the continuing yield losses by weeds and the effective weed control that helps to stabilize weed infestations and reduce yield losses (Chikowo et al. 2009). However, the use of herbicides is accompanied by environmental problems, such as contamination of food and environment by residues, development of herbicide-resistant weeds and shifts in the composition of weed populations (Beltran et al. 2012; Chikowo et al. 2009).

Integrated weed management

Integrated weed management (IWM) can be defined as a holistic approach to weed management that integrates different methods of weed control to provide the crop with an advantage over weeds (Harker and O'Donovan, 2012). IWM can decrease the density of weeds in crops, reduce the relative competitive ability of weeds such that it can help preserve crop yields and limit the size of the weed seed bank, and can control emerged weeds, reducing the need for herbicide application (Buhler 2005; Deytieux et al. 2012). Therefore, IWM may include natural process such as seed predation that can contribute and improve the weed management.

Weed seed predation

Seed predation is a plant-animal interaction in which granivores (seed predators) feed on the plant seeds as a main food source (Hulme and Bekman, 2002). Weed seed predation can reduce weed seed banks (Westerman et al. 2003) thereby limiting weed population density. In cropping systems, seed mortality by predation combined with other control tactics can lead to a decrease of herbicides inputs (Westerman et al. 2005; Williams et al. 2009).

In Netherlands, rodents predated 26-83% of weed seeds in sugar beet fields (Westerman et al. 2011) while rodents and invertebrates predated 32-70% of seeds in cereal fields (Westerman et al. 2003). Similarly, in Germany, the predation rate by invertebrates was low in winter wheat fields (Baraibar et al. 2012). Seed predation can occur prior to or after seed rain. Pre-dispersal seed predators consume seeds before seed shed by the parent plant (Harper, 1977). Post-dispersal seed predation causes seed loss that starts with seed shed and ends before seed burial (Westerman et al. 2003). This study is focused on post-dispersal seed predation rates by the predator *Messor barbarus* L. in dryland cereal fields in NE Spain.

Post-dispersal seed predation in dryland cereals

In arable fields in NE Spain, winter cereals are the main crops grown on non-irrigated land (MAPA, 2011). Current weed control is mainly chemical with additional practices such as tillage, delayed sowing date or increasing seeding rate (Gonzalez-Andujar and Fernandez-Quintanilla 2004). In this area, the harvester ant *M. barbarus* is the main seed predator. Seed removal rates by rodents are low throughout the season and the density of

carbides is low (Baraibar et al. 2009). Post-dispersal seed predation by harvester ants is high (78%) in spring and summer. However, factors that determine the temporal and spatial variability in predation rates are largely unknown. This knowledge could help to better understand the constraints and potentials of seed predation by harvester ants for weed management.

Temporal and spatial variability of seed predation

One characteristic of seed predation is that it is variable over time and space. For example, seasonal variability of seed predation in small grain cereals and sugar beet fields (Westerman et al. 2003, 2011) provide examples of temporal variability. Furthermore, influence of patch size (Orrock et al. 2003), and spatial arrangement of ant nests on seed predation rates (Díaz 1992; Azcárate and Peco 2003) provide examples of spatial variability. This variability can influence the efficacy of granivory and may provide opportunities to weeds to escape predation.

With regard to temporal variability, the degree of overlap between the main period of seed shed and the main period of activity of the seed predators determines the total percentage of seed predation over a year (Davis et al. 2011). If the seed predators are active when most seeds are being shed, it is likely that a large proportion of seeds will be consumed, unless they become satiated. If satiation plays a role, the proportion of seeds predated will decrease with increasing seed density (inverse density dependent response). However, for seed predators that can store or cache seeds, such as granivorous rodents or harvester ants, it is unlikely that they will become satiated easily. In fact, they may increase foraging activity with increasing seed density, leading to a direct density-

dependent response. If seed predators are active prior to or after most weed seeds have been shed, however, it is likely that a large proportion of seeds will escape predation.

Less is known with regard to the effect of spatial variability on total seed predation rate or opportunities to weeds to persist. Harvester ants of the species *M. barbarus* form large nests that usually stay in one place; relocation is rare (Lopez et al. 1993) and competition between nests usually leads to the elimination of one or more of the competitors (Diaz 1991; Wiernasz and Cole 1995). Competition increases with size of the colonies. Nest location is determined by the founding queens after the maiden flight and is largely random, except that young queens may be killed when they land in the territory of an established nest. The larger the nest, more workers are available for foraging. Consequently, nest size and nest location may be important determinants of granivory efficiency. These may interact with the spatial distribution of weed seeds. Weeds do not occur randomly in a field, but in patches. Consequently, seeds will occur in patches too. Interestingly, patch size and patch quality, in terms of seed density, may vary between fields and between weed species. It is largely unknown if and how patch size or patch density influence predation rates by seed predators. However, if predation rates differ between differently sized patches or patches of different quality, it is likely that this may again provide opportunities to weeds to escape predation.

The objectives of the current study were therefore to:

1) Estimate annual seed losses due to predation.

2) Quantify seed predation rates with increasing seed densities.

3) Quantify the effect of patch size on predation rates.

4) Determine the relationship between spatial nest arrangement and ant foraging intensity within a field.

Objective 1

Identifying the most vulnerable stages in the life cycle of a weed may help to design better weed management strategies. Seed survival, germination, seedling growth and survival are considered the most critical stages (Harper 1977, Schupp 1995). After seeds are shed from the mother plant, they land on the soil surface where they may develop into seedlings or be incorporated into the soil matrix. However, on the soil surface they may become victims of post-dispersal seed predators, while buried or covered seeds become inaccessible to predators (Westerman et al. 2009; Hulme 1994). The time until burial into the soil depends on seed characteristics, such as size, shape or awns (Peart 1979, Hulme and Benkman 2002). For example, larger seeds will be at greater risk from seed predators due to a lower burial rate (Westerman et al. 2009). The availability of seeds to predators may decrease during the harvest when seeds are covered by the dust, soil and straw produced (Westerman et al. 2006; Westerman et al. 2009). Seed predation risk will be the greatest if the timing of seed shed overlaps with the period of highest predator activity (Westerman et al. 2003; Westerman et al. 2011). To estimate the annual seed losses of weeds due the predation by ants, seed predation rates were measured as well as the timing of seed shed. Long-term seed losses due to predation were estimated using an existing model, which integrates short-term rates of seed shed, burial and removal (Chapter 2).

Objective 2

Weed populations have patchy distributions (Johnson et al. 1996; Dieleman and Mortensen 1999). The spatial distribution can be related to numerous interacting factors, such as soil type (Dieleman et al. 2000), type and frequency of cultivation (Colbach et al. 2000), seed dispersal during the harvest (Blanco-Moreno et al. 2004), herbicide type and efficacy (Dieleman et al. 2000) and crop interference (Inderjit and Weiner 2001).

Seed predators may eliminate weed patches if they respond in a direct density-dependent way, meaning that seed predation rates increase with seed density (Cromar et al. 1999; Baraibar et al. 2012). Predation rates may decrease with increasing seed density if predators respond in a inversely-density dependent way (Westerman et al. 2008), which will cause weed patch persistence. To determine if seed predation rates by harvester ants are influenced by the seed density in patches, seed patches varying in seed density were created in a cereal field after harvest. Seed removal rates were determined after exposure (Chapter 3).

Objective 3

The size of a weed patch may influence the probability that the patch is discovered and exploited by ants. Patch perimeter and area-to-perimeter ratio determine the probability of patch detection by predators (Orrock et al. 2003). Larger patches are expected to be encountered and exploited with a higher probability than small patches due to a larger perimeter and a larger area-to-perimeter ratio. Here, the relationships between weed patch size and patch detection and predation rates were established. Seed patches varying in

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size were created in a cereal field. After exposure, the remaining seeds were collected and used to estimate seed predation rates (Chapter 4).

Objective 4

Seeds that occur close to ant nests should suffer higher predation pressure than seeds further away because the foraging activity of harvester ants decreases with distance from the nest entrance (Azcárate and Peco 2003). An uneven distribution of ants could lead to variable foraging intensity in the field (Janzen 1971; Crist and Macmahon 1992). Variability in ant densities was estimated by studying the spatial distribution of nests in combination with nest size. When all nests are of equal size and arranged regularly over a field, seeds anywhere in the field have the same probability of being found, and exploited. When nests vary in size and the arrangement is overdispersed, the location of a seed may determine the probability of being found and exploited. Therefore, the spatial arrangement of *M. barbarus* nests was analyzed using spatial point processes (Chapter 5).

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CHAPTER 2

Differential weed seed removal in dryland cereals

Abstract

Weeds persist in rain-fed cereal fields in NE Spain, despite intense herbicide use and high seed removal rates by granivorous harvester ants. Herbicide resistance is involved, but certain weed species also appear to escape seed removal by granivores. To identify the mechanisms involved, we measured seed removal rates (three fields in 2010) and the timing of seed shed (one field in 2009 and three fields in 2010), and used an existing model, which integrates short-term rates of seed shed, burial and removal, to estimate long-term seed removal rates.

Averaged over years, fields and weed species the long-term seed removal rate was estimated at 72% (range 46-100%). Fifteen to 25% of the seeds of *Bromus diandrus* avoided removal by being less attractive (low removal rates), and another 0-29% escaped through crop harvest, which made seeds inaccessible to granivores. Similarly, 20-32% of the *Papaver rhoeas* seeds escaped through crop harvest, while another 13-17% escaped by burial into the soil (small seed size). Other species, such as *Galium spurium* or *Diplotaxis erucoides*, had no means of avoiding seed removal by harvester ants. In particular the more troublesome weeds, such as *B. diandrus*, *P. rhoeas* and *L. rigidum*, combined herbicide resistance or tolerance with avoidance mechanisms against granivory.

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Introduction

Seed removal (granivory) can contribute to weed control. When enough of the newly produced weed seeds are intercepted by granivores, seed reservoirs in the soil are slowly depleted, resulting in lower weed densities. Estimates of annual removal rates, however, are difficult to obtain experimentally, because both seed shed and seed predation are highly dynamic and erratic over a season, forcing measurements of weed seed shed and weed seed removal rates to be made at a daily of weekly time-scale. These episodic point estimates then have to be scaled to long-term removal rates (Davis et al. 2011). It is important to understand the factors that influence the estimates of annual removal rates, in order to predict annual removal rates and long-term effects on weed population dynamics, and to identify management practices that foster high levels of weed seed removal.

In temperate regions, differences in annual removal rates between weed species mainly stem from differences in the degree of overlap between the period of highest activity of granivores and the period that seed are available on the soil surface. For example, in phenologically early crops, such as small-grain cereals, the peak in seed demand by granivores precedes the peak in weed seed shed (Westerman et al. 2003), causing early-maturing weed species to sustain higher seed losses than late-maturing weed species.

Several weed species can cause substantial losses in cereal yields in NE Spain (Escorial et al. 2011; Torra et al. 2011). The presence of these species can partially be explained by resistance or differential susceptibility to herbicides. For example, *Papaver rhoeas* L. and *Lolium rigidum rigidum* (Gaud.) are resistant and *Bromus diandrus* Roth.

tolerant to several commonly used herbicides (Escorial et al. 2011; Heap 2012). However, for weed species to persist they also have to be able to evade seed predation. Seed removal rates are high all season long (Baraibar et al. 2009), and, therefore, weed seeds cannot escape seed predation because of dissimilar periods of seed shed and seed demand. So, how do these seeds avoid predation?

The main seed predator is a harvester ant, *Messor barbarus* L., whose activity is constrained by temperature and internal cues. Foraging activity is high as long as the surface temperatures are between approx. 15 and 35 °C (Azcárate et al. 2007), which is usually from early May until mid October (Generalitat de Catalunya 2011), with a temporary trough at the end of September or early October caused by colony reproduction (Baraibar et al. 2009). Other seed predators, such as carabid beetles and granivorous rodents, do occur, but their numbers are low (Baraibar et al. 2009), and their foraging activity negligible compared to that caused by the massive presence of harvester ants (Baraibar and Westerman, pers. obs.). The causes for the low numbers and low activity-densities are probably water- and food-shortage in the case of rodents (the area is semi-arid), and competition with harvester ants in the case of carabid beetles.

A particular seed species could avoid being collected if other seed species are more attractive, more nutritious, or easier to handle. Harvester ants tend to have clear preferences. In general, larger seeds with a soft seed coat are collected more frequently than smaller seeds with a tough seed coat (Willott et al. 2000; Reyes-López and Fernández-Haeger 2002a). Seed selection may further be influenced by the relative abundance of seeds (Willott et al. 2000) and the state of filling of the granaries, which are large subterranean storage rooms for seeds. The fuller the granaries, the smaller the seeds

collected (Reyes-López and Fernández-Haeger 2002b). It is unknown how various common species of weed seeds in NE Spain differ in attractiveness to harvester ants.

Weeds can also avoid predation by retaining their seeds in the seed heads until crop harvest; seed removal rates drop noticeably following crop harvest (Baraibar et al. 2009). Dust, soil and straw produced during harvest cover seeds upon dispersal, making them inaccessible to seed predators (Westerman et al. 2006, 2009). Plant debris hinders the movement and foraging of harvester ants (Atanackovic pers. obs.). Furthermore, the presence of waste grain, which is an attractive food source, distracts harvester ants from foraging on weed seeds. Once the straw is removed, the weed seeds become accessible again. However, in no-till fields, the straw is usually left on the field for 4-5 weeks, because there is no incentive or need to remove the straw, as fields are left fallow until seeding in late autumn. In conventionally managed fields, the straw is removed more quickly, but followed by tillage, which transports the seeds to deeper soil layers and out of reach of granivores. Either way, seeds shed at crop harvest have a much better chance of avoiding predation than seeds shed during the cropping season.

We hypothesized that certain weed species may suffer lower removal rates than others, if their seeds were 1) less attractive or 2) shed at crop harvest. To test our hypotheses, the timing of seed shed (one field in 2009 and three fields in 2010) and the pattern of seed removal over time (three fields in 2010) were determined in commercial cereal fields in NE Spain. Seed preference was estimated by comparing seed removal rates of four weed species in one of the fields in 2010. Next, an existing model was used to integrate the dynamics of seed shed and seed removal to estimate long-term removal rates (Westerman et al. 2003).

Materials and methods

Locations

Trials were conducted in commercial winter cereal fields in Agramunt (field 1 (41°46′12″N 1°6′2″E); clay soil) in 2009 and 2010, and Vilanova de Bellpuig (field 2 (41°35′23″N 0°58′23″E) and field 3 (41°35′24″N 0°58′41″E); sandy clay soil) in 2010. Details of crops, date of sowing and harvest, and other management details are provided in Table 2.1. The region is semi-arid; average annual temperature is 14.7°C (1971–2000; Agencia Española de Meteorología 2012), and average annual rainfall is 369 mm, concentrated in spring and autumn. Summers are hot (average max. 33°C) and winters mild (average min. 0°C).

Weed survey

On 8 May 2009, field 1 was surveyed using 30 randomly selected 0.4 m^2 (0.4 ×1.0 m) quadrats, which were placed between crop rows in the areas selected for seed shed measurements (see below). Weeds were identified to species level and densities were expressed as numbers per m² (Table 2.2). On 17 June 2010, all three fields were surveyed as described for 2009, using 20 random 0.4 m^2 quadrats in fields 2 and 3, and 25 0.4 m^2 quadrats in field 1, because weed diversity tended to be higher (Table 2.2). Occasionally, a rare plant species was encountered, i.e., one or two individuals per fields, but these were omitted. The size of the dispersal units, i.e., seeds with hulls, fruits or other structures naturally attached to the seed (except awns or hairs), were determined for 18 seeds per species, using an ocular micrometer.

Table 2.1 Crop management in the fields 1, 2 and 3 in NE Spain, in 2009 and 2010; location, crop type, dates of sowing, harvest and straw removal, and details on herbicide applications

Field	Size [ha]	Location	Crop	Row dist. [cm]	Date of			Herbicide application [g ai ha^{-1}]			
					sowing	harvest	straw removal	Pre-emergence	date	Post-emergence	date
2009											
1	5.4	Agramunt	barley	18	25-10-08	22-6-09	25-7-09	glyphosate (720) + mesosulfuron-methyl (1200)	18-10-08	No herbicides	-
2010											
1	5.4	Agramunt	wheat, triticale	18	23-10-09	25-6-10	30-7-10	glyphosate (720) + mesosulfuron-methyl (1200)	16-10-09	No herbicides	-
2	2.1	Vilanova de Bellpuig	barley	20	29-10-09	25-6-10	25-7-10	glyphosate (540) + MCPA* (200)	25-10-09	florasulam (22) + 2,4-D** (1050)	30-1-10
3	6.1	Vilanova de Bellpuig	barley	20	29-10-09	25-6-10	25-7-10	No herbicides	-	florasulam (22) + 2,4-D** (1050	30-1-10

* 2-methyl-4-chlorophenoxyacetic acid; **2,4-dichlorophenoxyacetic acid

Table 2.2 Densities for the main weed species found in fields 1, 2 and 3, in NE Spain, in 2009 and 2010 (plants m^{-2}; mean ± SE). Data for 2009 is based on 30, 0.4 m² quadrats (0.4 × 1.0 m), and for 2010 on 25, 20, and 20, 0.4 m² quadrats in fields 1, 2 and 3, respectively.

	Density (mean ± SE) [m^{-2}]			
Year	2009	2010		
Weed species	1	1	2	3
Field				
Galium spurium	7.2 ± 1.7	1.6 ± 0.4	-	-
Bromus diandrus	99.1 ± 8.8	9.2 ± 3.3	-	-
Lolium rigidum	0.2 ± 0.1	-	1.6 ± 1.0	9.4 ± 1.1
Avena sterilis	-	-	3.6 ± 0.6	20.3 ± 2.7
Diplotaxis erucoides	-	-	-	27.9 ± 4.4
Papaver rhoeas	35.5 ± 5.5	24.6 ± 2.7	-	-
Chenopodium album	2.8 ± 1.0	11.3 ± 2.0	2.3 ± 1.4	-
Anacyclus clavatus	17.2 ± 2.6	-	4.1 ± 1.0	-
Kochia scoparia	-	4.8 ± 1.4	-	2.4 ± 2.4

Weed seed shed

In each field, an area of 50 × 50 m was selected at least 5 m from the nearest field margin. Thirty-six seed traps were placed in each area, organized in nine transects with four traps per transect. In the case of field 3, a 25 × 100 m area was selected, because not the entire area had been treated with herbicides in October 2009, causing weed densities to be uncharacteristically high in 2010. The selected area was such that it fit inside the herbicide-treated area. Here, traps were placed along two transects with 18 traps per transect. The distance between transects was always 5 m and between traps within transects 10 m. Traps were emptied once per week and collected seeds were stored in plastic bags until further processing. In the case of excessive rainfall, the contents of the traps were poured through a sieve and the seeds dried in laboratory for 4 hours at 40 ºC.

Measurements started on 29 April in 2009 (field 1) and on 13 April in 2010 (all fields), prior to the onset of weed seed shed. The experiment was terminated shortly after harvest on 22 June 2009 (field 1) and 27 June 2010 (all fields). Crop harvest stopped weed seed production altogether; weed plants were either cut during harvest, or smothered by the straw left on the field. Soil moisture after harvest is usually insufficient to induce another flush of weed seedlings.

Seed traps were modified after Westerman et al. (2003), and consisted of two aluminium trays (25 × 13 × 3 cm; l × w × h; total trap surface 0.0645 m²) placed next to each other between two crop rows on the soil surface and fixed in position by nails. Each trap was covered by small-mesh (6 mm) metal netting to keep out vertebrates. The external walls of the trays were coated with fluon (Polytetrafluoroethylene, BioQuip Products Inc. Rancho Dominguez, CA, USA) to keep out insects. To measure seed shed during harvest, traps were dug into the soil a few days before harvest, such that the top of each trap was flush with the soil surface. Traps and their contents were retrieved immediately after harvest. Seeds of most species entered the traps without difficulty. However, bigger seeds, such as those of *Avena sterilis* L. and *B. diandrus*, accumulated on the mesh on top of the trays and these seeds were carefully collected and included in the seed samples. It is possible that some seeds had already been gathered and consumed by seed predators.

Seeds collected were identified and counted. The average number of seeds caught per m² per week, *Y*, and the total number of seeds per m² for the entire season were estimated for each weed species. To match seed shed with the seed removal rate, seed shed per two days, Y_i, was calculated as $Y \times 2/7$ (m^{-2}) for each 2 d period from mid April

until and including harvest, assuming that the daily seed shed was constant within each collection period.

Weed seed removal rate

Seed removal was measured once per month from April to June 2010, as the percentage of seed removal per two days averaged over 25 feeding stations per weed species per field. Farmers would not allow trials to interfere with management and, therefore, seed removal rates could not be determined after crop harvest. As with seed shed, seed removal was measured in 50×50 m areas. All areas were at least 2 m away from the selected areas in which seed shed was measured and 5 m away from the nearest field edge. Because of its unusual shape and the fact that it had to accommodate four times as many feeding stations as the other fields, two areas were selected in field 1; 1a (50×90 m) and 1b (50×30 m). Forty feeding stations were placed in area 1a (40), organized in four rows and ten columns, and 60 stations were placed in area 1b, organized in six rows and ten columns. In fields 2 and 3, 25 feeding stations were organized along a regular grid of five rows and five columns. The distance between feeding stations was always 10 m. The experimental unit is the feeding station.

In field 1, 25 feeding stations each were filled with 2 g of *Lolium rigidum* (Gaud.) (2.59 ± 0.043 mg seed[-1], Herbiseed, Reading, UK), *Papaver rhoeas* L. (0.0118 ± 0.002 mg seed [-1], Herbiseed, Reading, UK), *Galium spurium* L. (0.61 ± 0.007 mg seed,[-1] Herbiseed, Reading, UK) or *Bromus diandrus* Roth (13.183 ± 1.1 mg seed [-1], collected in Agramunt in 2009) to allow testing for seed preference. *Lolium rigidum* is readily taken by harvester ants and other seed predators (Baraibar et al. 2009) and is naturally present

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in the area. The other species were among the most abundant weed species in field 1 in 2009. The distribution of seed species was chosen randomly from the available positions in areas 1a and 1b. In fields 2 and 3, feeding stations were filled with 2 g of *L. rigidum*. All seeds were dried for 4 h at 40 °C prior to weighing, to standardize seed weight.

Each feeding station consisted of one half of a plastic, 9-cm diameter Petri dish with two 15 mm wide openings in the sides to facilitate entry to harvester ants and other invertebrates. Previous studies had indicated that two openings were more than sufficient to allow full utilization of the available seeds (Díaz 1992; Baraibar et al. 2009). Dishes were covered by 1 cm mesh metal cages (10 × 11 × 3 cm) and served to keep out vertebrates, if any. Feeding stations were installed on 13 April, 26 May and 25 June 2010. Two days later, the remaining seeds were retrieved, dried, and weighed. Seed removal rates were calculated as the weight of seeds removed per two days, relative to the initial amount provided. Seed removal rates for each two-day interval from April-June were obtained via linear interpolation.

Statistical analysis

The weight of seeds recovered per feeding station was analysed to test for the effect of weed species and sampling date on seed removal rate in field 1 in 2010. A regression model (GLM) was used with a logit link and a binomial variance function allowing for overdispersion (Genstat 11; Genstat 5 Committee 1993). Significance was evaluated in terms of mean deviance ratios, which were, in turn, evaluated by comparison with *F*-distributions ($\alpha = 0 \cdot 05$). For significant effects, the t-test was used to rank means.

Similarly, the weight of *L. rigidum* recovered per feeding station in all three fields in 2010 was analysed to test for differences between fields.

Long-term seed removal rates

Many weeds have extended periods of seed shed. As a result, seeds that are produced on weed plants become available to epigaeic seed predators gradually. The longer seeds are exposed on the soil surface, the higher the probability that they will be consumed by granivores. Once covered by soil or debris, they have a very low probability of being found and consumed. The speed with which seed are incorporated into the soil matrix is influenced by, for example, size, shape and soil conditions (Westerman et al. 2009). Most weed seeds tend to be quickly buried due to their small size. Because of the differential timing of seed shed and seed burial, each seed cohort will be exposed to granivores for a different period of time during the season. At the same time, seed removal rates vary over the season, resulting in cohort-specific seasonal predations rates. Ergo, granivory over an entire season cannot be measured directly as the proportion of seeds removed at the end of the season, but has to be calculated as the mean of predation rates sustained by each of the seed cohorts.

A model that combines information on the timing of seed shed, differences between weed species in the duration of seed exposure, and seasonal variability in seed removal by granivores is available to estimate seasonal predation rates (Westerman et al. 2003). It follows the fate of seeds in each cohort (consumed, buried or on the soil surface) over time and seed losses per cohort are combined into a single estimate, the long-term removal rate ($\overline{M}$), via a weighted mean, with cohort size as the weighting factor.

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$\overline{M}$ was calculated via $\overline{S}$, the proportion of newly produced seeds that survives

removal; $\overline{M} = 1 - \overline{S}$, with

$$\overline{S} = \frac{\sum_{i=1}^{n}\left(Y_i\prod_{j=i}^{k}S_i\right)}{\sum_{i=1}^{n}Y_i} \tag{1}$$

The denominator in (1) is the total number of seeds produced over the entire period and

the numerator the total number of seeds not removed by predators. The latter is calculated

by subdividing seed production into n 2-day cohorts of size Y_i and following their fate

over time. Survival of each seed cohort was calculated as the product of survival chances

per two days, $S_i = 1 - M_i$, during the k, 2-day periods that seeds are exposed to seed

predators on the soil surface, with M_i the seed removal rate for the ith time period. For

further details on the model, we refer to Westerman et al. (2003). The implicit assumption

to be able to use of data gathered in this study in model calculations is that proportions

based on weight are equivalent to proportions based on numbers. It was furthermore

assumed that both seed shed and seed removal ended immediately after crop harvest. The

model was therefore adapted to $\overline{M}_h = 1 - (\overline{S}_h + H)$, where $\overline{M}_h$ is the long-term removal rate

from the onset of seed shed until and including crop harvest, $\overline{S}_h$ the proportion of seeds

not removed by granivores between the onset of seed shed and crop harvest, and H the

proportion of seeds shed during harvest. Thus, $\overline{S}_h + H$ serves as an estimate of the long-

term seed survival rate, $\overline{S}$. Species with a peak in seed shed that coincides with crop

harvest will have a large H; species with a low preference by harvester ants (low removal

rate) will have higher estimates of $\overline{S}_h$. Species-specific, long-term removal rates, $\overline{M}_h$ were

calculated for those species, for which both species-specific seed shed and seed removal

data were available, namely for *P. rhoeas*, *G. spurium*, and *B. diandrus* in field 1 in 2009 and all four weed species in 2010, and *L. rigidum* in fields 2 and 3 in 2010.

Unfortunately, one of the variables in equation 1, namely the mean duration of exposure of seeds on the soil surface ($k \times 2$ d), was unknown. The exposure time is directly related to seed size; the bigger the seed, the slower the rate of incorporation into the soil matrix. Using a rough extrapolation of the data by Westerman et al. (2009), the mean exposure time for *P. rhoeas* seeds (seed size 1.0 mm) would be 8 d, for *G. spurium* 10 d (1.1 mm), and for *B. diandrus* (length 21.2 mm; width 1.8 mm) and *L. rigidum* (length 6.6 mm; width 1.0 mm) 4 w. Probably, true exposure times were longer, because soils in NE Spain tend to form hard, dry crusts in summer, with little or no cracks. To test the sensitivity of $\overline{M}_h$ to the exposure time, $\overline{M}_h$ was calculated using the removal rates for three of the weed species in field 1 in 2010, while varying the duration of exposure from 2 d after seed shed $(k = i)$ to continuous exposure ($k = n$); i.e., as if seeds could shrink and expand. These preliminary calculations showed that $\overline{M}_h$ increased with exposure period until it levelled off after 6 (*G. spurium, P. rhoeas*) and 10 d (*B. diandrus*)(Fig 2.1). Seed removal was so fast that maximal removal ($1-(\overline{S}_h+H)$) was achieved within 10 d, which corresponds to a seed size of approx. 1 mm. For all seeds larger than 1 mm, the estimate of $\overline{M}_h$ will change little or nothing whether the exposure time was 10 d or longer, because seed burial rate (0.04 2d^{-1}) could not compete with seed removal rate (between 0.2 and $\approx$1.0 2d^{-1}). Only in the case of very small seeds (< 1 mm, e.g., *P. rhoeas*), could seeds escape into the subsoil (0.17 2d^{-1}) before they could be removed by ants. Calculations of $\overline{M}_h$ were, therefore, conducted with the appropriate exposure times as estimated above. 2010).
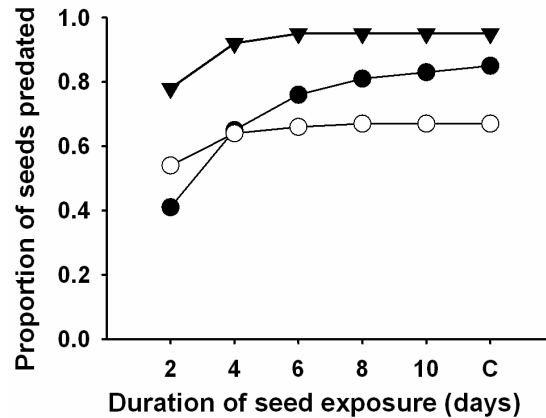
Fig 2.1 Proportion of annual seed loss due to removal, $\overline{M}_h$, as a function of the exposure time in field 1 in 2010 for *Bromus diandrus* (●), *Papaver rhoeas* (○) and *Galium spurium* (▼). 'C' refers to continuous seed exposure.

Results

Weed seed shed

The timing of seed shed and the number of seeds produced differed between weed species and fields. Seed shed of *Diplotaxis erucoides* (L.) DC. started and peaked early, while that of *P. rhoeas*, *B. diandrus*, and *A. sterilis* started and peaked late (Fig 2.2). No seeds of *Chenopodium album* L. *Anacyclus clavatus* (Desf.) Pers.*,* or *Kochia scoparia* (L.) Roth were collected, despite the fact that these species had been present (Table 2.2). Seeds of *A. clavatus* were unripe and retained in the seed heads, most plants of *K. scoparia* were seedlings or small, vegetative plants, and plants of *C. album* remained vegetative.

Seed removal by granivores

Regression analysis showed that in field 1, the rate of seed removal increased significantly over time ($p = 0.04$) and did so differently for the four weed species (weed species, $p < 0.001$; interaction weed species × sampling date, $p < 0.001$). In May 2010 a significantly higher proportion of *G. spurium* (0.7±0.08) and *L. rigidum* (0.9±0.05) and in June, a significantly higher proportion of *L. rigidum* (1.0±0.00) and *P. rhoeas* (0.9±0.06) were removed, compared to the other two species (Fig 2.3A). In June, the proportion seed removal of *B. diandrus* (0.2±0.07) was significantly lower than that of *G. spurium* (0.6±0.09). Overall, *B. diandrus* was the least and *L. rigidum* the most preferred seed species (Fig 2.3A).

Seed removal rates of *L. rigidum* increased significantly over time ($p < 0.001$)(Fig 2.3B), but did not differ between the three fields ($p = 0.94$), indicating that the estimates were consistent over space. Furthermore, seed removal rates in this study corresponded reasonably well with those obtained for *L. multiflorum* by Baraibar et al. (2009, 2011), suggesting that seed removal patterns are consistent over time as well.

Long-term seed removal rates

Averaged over years, fields and weed species the long-term seed removal rate, $\overline{M}_h$, was estimated at 72% (range 46-100%) (Table 2.3). $\overline{M}_h$ was highest for *G. spurium* in both years and lowest for either *P. rhoeas* or *B. diandrus*. Estimates of $\overline{M}_h$ varied between years, causing the ranking of $\overline{M}_h$ for weed species to differ between years as well.

Long-term seed removal rate for *D. erucoides* and *A. sterilis* could not be properly estimated, because no species-specific removal rates had been determined. However,

using average removal rates over all four weed species in field 1 and an exposure time of 14 d for *D. erucoides* (seed size 1.3 mm) and 4 w for *A. sterilis* (seed length 15.6 mm; width 2.0 mm), $\overline{M}_h$ would have been 100% in either case.

The proportion of seeds shed at crop harvest

In field 1, the proportion of seeds shed during harvest, H, varied from 0 to 0.32 in 2009 and from 0 to 0.40 in 2010 (Table 2.3). Weed species that dispersed a large proportion of their seeds during harvest were *L. rigidum* ($H = 0.25\text{-}0.40$) and *P. rhoeas* ($H = 0.20\text{-}0.32$). In contrast, *G. spurium* in 2009 ($H = 0$), and *G. spurium* ($H = 0.03$), *B. driandrus* ($H = 0$)*, D. erucoides* ($H = 0$), and *A. sterilis* ($H = 0.09$ and 0.05) in 2010 shed all or almost all of their seeds prior to crop harvest.

Table 2.3 Seed production ($\sum_{i=1}^{n} Y_i$) and the estimated fate of seeds of *Papaver rhoeas*, *Galium spurium*, *Lolium rigidum* and *Bromus diandrus* in field 1 in 2009 and 2010; with $\overline{M}_h$, the proportion of seeds removed up to the moment of crop harvest; $\overline{S}_h$, the proportion of seeds not removed up to the moment of harvest; H, the proportion of seeds shed at harvest; and $\overline{S} \approx \overline{S}_h + H$, the long-term seed survival rate.

Weed species	$\sum_{i=1}^{n} Y_i$ [m^{-2}]	$\overline{M}_h$	$\overline{S}_h$	H	$\overline{S} = \overline{S}_h + H$
Field 1; 2009					
Papaver rhoeas	84604	0.51	0.17	0.32	0.49
Galium spurium	59	1.00	0.00	0.00	0.00
Lolium rigidum	114	0.67	0.02	0.31	0.33
Bromus diandrus	4089	0.46	0.25	0.29	0.54
Field 1; 2010					
Papaver rhoeas	112130	0.67	0.13	0.20	0.33
Galium spurium	248	0.95	0.02	0.03	0.05
Bromus diandrus	36	0.85	0.15	0.00	0.15
***Lolium rigidum*; 2010**					
Field 2	56	0.60	0.00	0.40	0.40
Field 3	598	0.75	0.00	0.25	0.25

The effect of seed preference

The lower seed removal rates obtained for *B. diandrus* (Fig 2.3A) translated into higher estimates of $\overline{S}_h$ (0.25 for 2009 and 0.15 for 2010) (Table 2.3) than for any of the other species. *P. rhoeas* also had relative high estimates of $\overline{S}_h$ (0.17 for 2009 and 0.13 for 2010) (Table 2.3). However, these did not originate from lower seed removal rates, but from a higher seed burial rate, which is equivalent to a shorter exposure period.
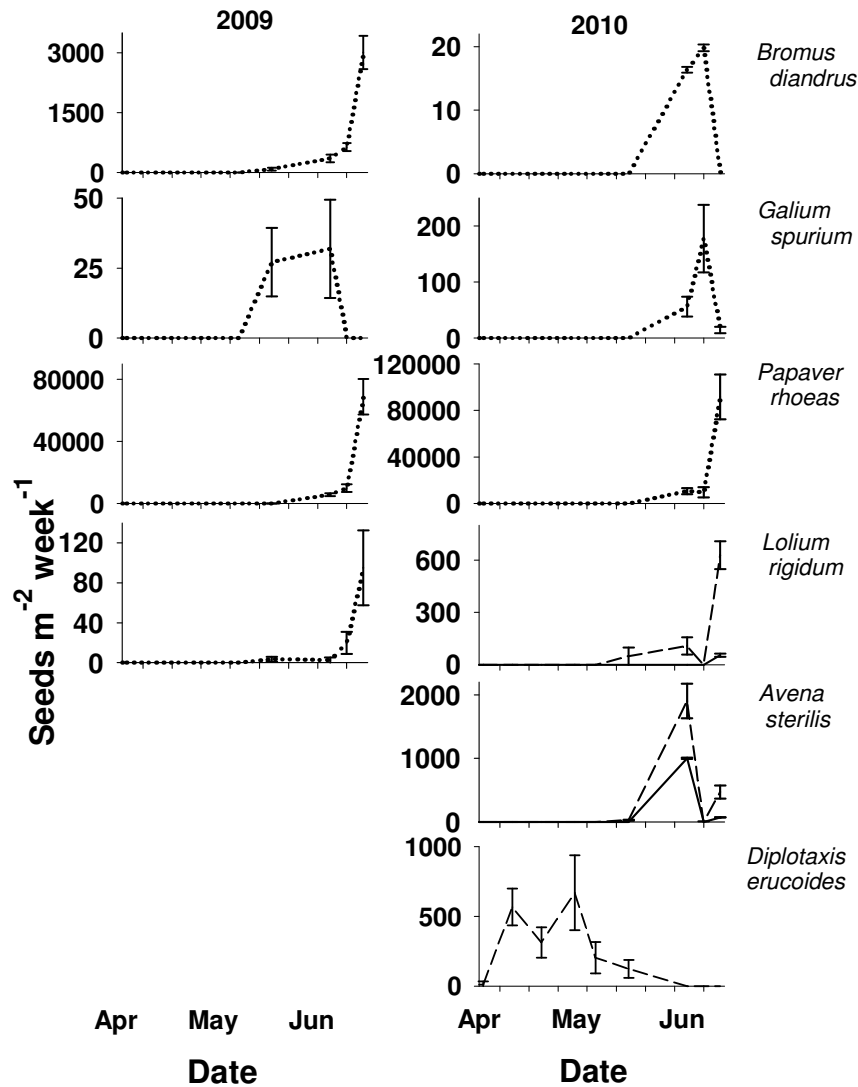
Fig 2.2 Weed seed production (seeds m⁻² week⁻¹) as measured in 36 seed traps per selected area, each consisting of two aluminium trays (25 × 13 × 3 cm; l × w × h; total trap surface 0.0645 m²), in field 1 (·····) field 2 (——) and field 3 (– – –) in 2009 (left side) and 2010 (right side), for *Bromus diandrus*, *Galium spurium*, *Papaver rhoeas*, *Lolium rigidum Avena sterilis* and *Diplotaxis erucoides*. Bars represent standard errors.
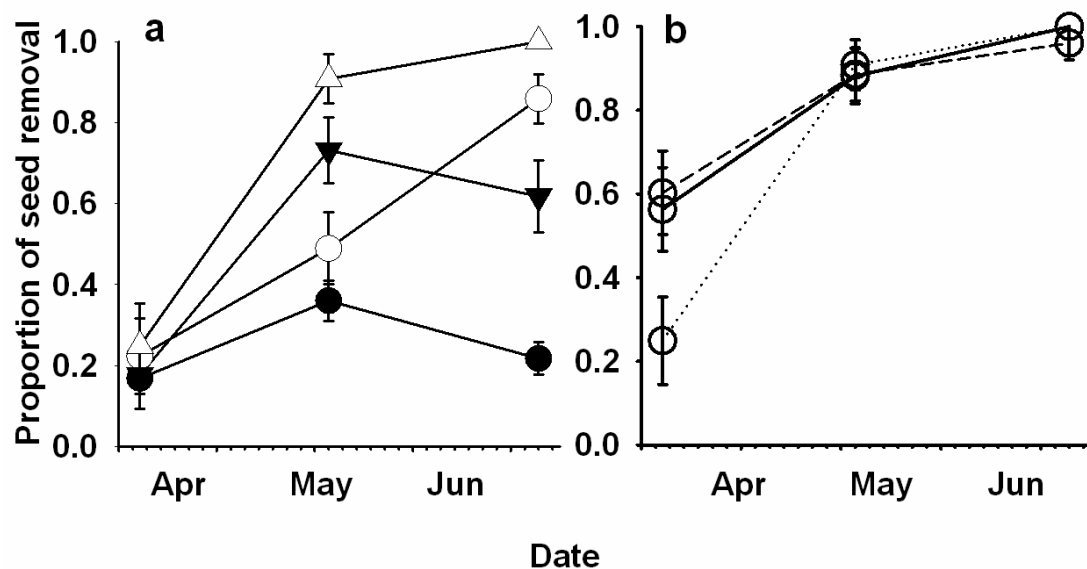
Fig 2.3 Seed removal rate (proportion seeds 2-days^{-1}) as measured in 25 feeding stations per weed species per field for *Bromus diandrus* (●), *Papaver rhoeas* (○), *L. rigidum* (Δ) and *G. spurium* (▼) in field C (A) for *Lolium rigidum* in field A (– –), field B (—) and field C (···) (B), and in April, May and June 2010. Bars represent standard errors.

Discussion

Crop harvest created good opportunities for weed seeds to escape removal by harvester ants. The proportion of seeds shed at harvest, *H*, depended solely on the shape of seed dispersal curve relative to the timing of crop harvest. Differences in the estimates of *H* between years were, therefore, caused by differences in the phenology of the weeds and in harvest date. Advancing or delaying harvest by a few days could have a major impact on the value of *H*. For example, had crop harvest in 2010 occurred a week earlier, a large proportion of the seeds of *B. diandrus*, *G. spurium* and *A. sterilis* would have been shed during instead of prior to harvest (Fig 2.2). However, the timing of crop harvest will have little influence on *H* of, for example, *D. erucoides*, because the peak in seed shed of this

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weed species occurs in May, which is too far off from the date of crop harvest. Only weed species, whose phenology is more or less synchronised with that of the crop, such that the timing of weed seed shed is close to crop harvest, may escape predation via crop harvest. Similarly, the timing of crop harvest will not influence H of *P. rhoeas* either, but for entirely different reasons. The capsules that contain the ripe *P. rhoeas* seeds are constructed in such a way that they will retain (most) seeds, as long as they are kept in an upright position. Seeds will be shed when the capsules are upturned, such as during harvest.

Part of the newly shed seeds may still fall victim to granivores, because removal rates, although reduced, were not zero after crop harvest (Baraibar et al. 2009), consequently H was probably overestimated. Furthermore, the straw that covered the seeds was removed after 4-5 w. Seeds that had not disappeared into the subsoil by then could fall victim to harvester ants again. The estimates of H will be more accurate if crop harvest is immediately followed by tillage. Tillage moves seeds to deeper soil layers and out of reach of harvester ants. Depending on the implement used, only about 0.03-40% of the seeds will remain on the soil surface (e.g. Mohler et al. 2006; Spokas et al. 2007).

Low preference also provided opportunities to weed species to avoid removal by harvester ants. Seeds of *B. diandrus* were not preferred by harvester ants, as evidenced by much lower 2-days removal rates compared to the three other weed species (Fig 2.3A). The low removal rates bought the seeds enough time to disappear in the subsoil or to remain on the soil surface untouched by harvester ants until crop harvest. This resulted in relatively high estimates of $\bar{S}_h$ for *B. diandrus*. It is unknown why seeds of *B. diandrus* were not preferred; they should be attractive because they are large seeds with soft seed

coats. Maybe they had a low nutritional value or contained toxic secondary metabolites, maybe they were difficult to transport because of chaff and awns (Hulme and Benkman 2002). Harvester ants were frequently observed removing the awns before transportation of the seeds. Larger workers with stronger jaws were required for this job (B. Baraibar and P.R. Westerman, pers. obs.), thus limiting foraging efficiency.

The mechanism that is usually responsible for preventing seed removal by granivores in temperate regions, namely seed burial into the soil matrix (Westerman et al. 2006, 2009), was largely ineffective in NE Spain; removal rates by harvester ants were simply too high. Only in the case of *P. rhoeas* were seeds small enough that the burial rate could compete with the removal rate. A certain proportion of seeds of *P. rhoeas* could escape into the soil matrix before harvester ants could gather them. This too resulted in relatively high estimates of $\overline{S}_h$.

Given their size, the mean exposure time on the soil surface of *A. sterilis* seeds should at least be 4 w, which should result in complete removal by harvester ants. However, many grass species, including *A. sterilis*, have a hygroscopically-active awn, which propels the seed into cracks and indentions in the soil (Peart 1979). With every wetting-drying cycle, such as occurs during the early morning hours, a seed can move a centimetre or more from its original location. Once stuck in an indention or crack, the seed is anchored firmly on the microsite (Peart 1979) and may even propel itself into a crack, if present (Westerman, pers. obs.). The hygroscopically active awn could, therefore, constitute another way that seeds could avoid removal by harvester ants, simply by disappearing into the subsoil. However, this possibility needs to be confirmed in the field.

Over all weed species, fields and years, the long-term weed seed removal rate, $\overline{M}_h$, averaged 72% (range 46-100%). With these values, seed removal in NE Spain is among the highest ever recorded on arable fields. Similar rates have only been obtained for harvester ants in natural systems, such as deserts (e.g. Whitford 1978). As suspected, weed species that were currently difficult to control chemically because of herbicide resistance or tolerance, such as *B. diandrus*, *P. rhoeas* and *L. rigidum*, also employed one or more strategies to avoid, escape or prevent removal by harvester ants. Nevertheless, estimates of $\overline{M}_h$ still ranged from 46 to 85% for *B. diandrus*, 40-75% for *L. rigidum* and 51-67% for *P. rhoeas*. This suggests that harvester ants will eliminate at least an important part of these troublesome weeds. Apart from *G. spurium*, not many 'non-problematic' weeds were included in this study. However, the estimate of $\overline{M}_h$ for *G. spurium* (95-100%) suggests that such species could be fully controlled by harvester ants and other granivores.

In general, weeds, which have seeds larger than 1mm, which are preferred by granivores, and which have the peak in seed dispersal some time before crop harvest run the biggest risk of losing all newly produced seeds to granivores in rain-fed cereals in NE Spain. Losing or eliminating harvester ants from these fields would increase problems with the control of *B. diandrus*, *L. rigidum* and *P. rhoeas*, and would cause many more weed species to become problematic and difficult to control.

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CHAPTER 3

Density independent weed seed removal by harvester ants in

dryland cereals

Abstract

In arable fields, the type of response of seed predators to varying seed densities within weed patches could determine whether weed patches persist in the field or not, may playing a key role in weed population dynamics. In rain-fed cereals in NE Spain, the harvester ant, *Messor barbarus* L., is responsible for 46-100 % of seed removal. In this study, we investigated if seed predation rates by harvester ants are influenced by the seed density in patches. Therefore, 50 circular areas of 1 m² were created inside each of four, 50 x 50 m blocks in a cereal field after harvest. Blocks were seeded in succession between July and August. Each of ten patches received *Lolium multiflorum* L. seeds at 1000, 2000, 5000, 10000 or 20000 seeds m^{-2}, randomly. After 24 h, the remaining seeds were collected, using vacuum cleaners, to estimate seed removal rates. Harvester ant nests in each block were counted as an estimate of population density.

In three blocks, seed removal was extremely high irrespective of the seed density applied (99-100%). In these blocks nest densities ranged from 468 to 900 nest ha^{-1}. In the block where nest density was lowest (284 nests ha^{-1}) seed removal ranged from 82 to 99.9%, and was slightly lower in the low density patches. Consequently, seed removal rate was not influenced by seed density. The lack of response was mainly caused by the fact that removal rates were extremely high, caused by high ant nest densities.

Introduction

In arable fields, weed seed predation by granivores, such as insects, rodents or birds, can reduce the build-up of the seed bank leading to a lower pressure of annual weed species in subsequent years (Westerman et al. 2009). Weed densities vary within arable fields, forming areas with higher plant densities than elsewhere, the so-called weed patches. The formation and stability of these patches is variable in space and time as a consequence of differential population increase and spread in the crop field (Heijting et al. 2007). Several factors are known to influence patch location and density, such as natural and anthropogenic mechanisms of seed dispersal (harvesting or tillage (Blanco-Moreno et al. 2004; Barroso et al. 2006), spatial variation in seed bank density (Izquierdo et al. 2009) and spatial variability in weed control measures (Barroso et al. 2004). In addition, spatial variation in post dispersal seed losses may contribute to weed patch survival and growth (Cabin et al. 2000).

The effect of seed predators on weed population dynamics will be largest if they eliminate weed patches, which will only occur if seed predation rates increase with seed density (direct density dependence). Alternatively, if predation rates decrease with increasing seed density (inversely density dependence) weed patches will persist and continue to grow.

A direct density-dependent response was found for seeds of *Echinochloa crus-galli* (L.) P. Beauv. and *Chenopodium album* L. in corn fields (Cromar et al. 1999) and for *Lolium multiflorum* L. in winter cereal fields in Germany (Baraibar et al. 2012). An inversely density-dependent response was found for *Setaria faberi* Herrm. in corn fields

in USA (Westerman et al. 2008). A density independent response was found for seeds of four broadleaf weed species in soybean fields in USA (Brust and House 1988).

In dryland cereals in NE Spain, the main seed predator is the harvester ant *Messor barbarus* L. (Baraibar et al. 2009). They can cause up to 46-100% losses of seeds of troublesome weed species, such as *Bromus diandrus* Roth., *Lolium rigidum* (Gaud) and *Papaver rhoeas* L. (Westerman et al. 2012). In natural ecosystems, the response of *Messor* spp. to different seed densities is variable. For example, *M. ebeninus* and *M. andrei* respond in a direct density-dependent way (Avgar et al. 2008; Brown and Gordon 2000), *M. capitatus* and *M. arenarius* do not (Albert et al. 2005; Wilby and Shachak 2000). There is no information on the type of response of *M. barbarus* to seed patches in arable fields.

Our goal was to determine if seed removal rates increased or decreased with increasing levels of weed seed availability, because direct density dependent seed removal by the harvester ants could contribute to the elimination of weed patches in rain-fed cereals in NE Spain.

Material and methods

A trial was conducted in a commercial no-till field in Villanova de Bellpuig (41° 35′ 25.76″ N, 0° 58′ 36.28″ E, sandy clay soil) in NE Spain in 2009. The experimental area (200 × 50 m) was divided into four blocks (A, B, C and D) of 50 × 50 m each, and at least 20 m away from the field margin. Sixty five patches of 1 m² were established randomly in each block. Fifty patches were fully exposed to ants (exposed patches) and were used to obtain data on seed removal by predators; five patches (control patches) were used to

obtain information on the density of seeds naturally available on the soil surface before artificial seed application; the remaining 10 patches were used to determine the efficacy of the seed retrieval methodology (efficacy patches). Experiments started a month after oats harvest, and continued in succession, namely on 22 July in block A, 4 August in B, 7 August in D, and 21 August in C. The blocks functioned as four replications in time. Patches were created by removing the straw, then raking and cleaning the rest of the crop residues from the soil surface in a circle area of 1 m², as determined by a 0.56 m rope attached to a stick in the center of the circle.

L. multiflorum seeds (4.12 ± 0.03 mg 1000 seeds^{-2}; Semillas Batlle, Bell-Lloc, Spain) were used here because they are readily taken by harvester ants (Baraibar et al. 2009). Seeds were dried 4 hours in the oven at 40˚ C, and five weed densities were determined by weight: 4.12 g for 1000, 8.24 g for 2000, 20.6 g for 5000, 41.2 g for 10000 and 82.4 g for 20000 seeds m⁻².

After application, seeds were retrieved using suction devices; a D-Vac (Vortis; Burkard manufacturing Co. Ltd., 2001) and the vacuum cleaners, namely V_1 (PALSON, VC 366E-8, 1800 W) and V_2 (BLUESKY, BVC356_8, 1800 W). Anything on the soil surface was collected by vacuuming the patch area and emptying the contents of the internal containers into paper bags. The samples consisted of seeds, dry soil particles and small pieces of plant debris. Seeds were separated from soil by elutriation (Wiles et al. 1996; Westerman et al. 2008), dried at 40˚ C, and sieved and cleaned from debris manually. Seeds were weighed to estimate the number of seed retrieved; this was used to calculate the proportion of seed removal. The electricity for both vacuum cleaners was

generated by a power generator (BENZA, E4200, and 3.2 kW). The energy for the D-vac came from a self diesel engine.

Efficacy patches were used to determine the effect of seed density and the type of suction device used on seed recovery. For this purpose, seeds were uniformly applied by hand on the surface of the ten randomly selected efficacy patches in each block (two per seed density per block), one hour before seed application in the 'exposed patches'. Seeds were retrieved immediately after application, such that ants had no time to collect them. Details on which suction devices were used in which block and which seed density are provided in Table 3.1.

To assess the density of seeds naturally available on the soil surface before applying seeds of *L. multiflorum*, five control patches in each block were sampled a day before seed application, using vacuum cleaner V_1 in block A, C and D, V_2 in block B, C and D and the D-vac in all blocks. Soil samples were collected in paper bags and processed as described above. *Avena sativa* L. seeds were found with high variability, namely 34 ± 33.8 seeds m^{-2} in block A, 88 ± 29.9 seeds m^{-2} in block B, 14 ± 3.0 seeds m^{-2} in block C and 47 ± 15.8 seeds m^{-2} in block D.

Seed removal by harvester ants was determined in the fifty fully exposed patches per block. Each density of seeds was uniformly applied by hand with gloves on the surface of each of ten randomly selected patches in each block, early in the morning. Immediately after application, a team of five people recorded the time needed by ants to detect each patch, which was defined as the moment one or more ants were probing or carrying seeds in the patch. Each person checked 10 neighbouring patches for a period of 1 hour. The time needed to detect the patch was rounded to the nearest minute. One day

after application, the remaining seeds were collected. Two or three teams were working simultaneously with different suction devices to retrieve the seeds as fast as possible to ensure the same exposure time for all patches. Because the vacuum cleaners were operated for 4-6 minutes and the D-Vac for one minute, the numbers of patches cleaned by a particular device varied from block to block (Table 3.2). Seeds were weighed to estimate the number of seeds retrieved as described above. This was used to calculate the proportion of seed removal. Patches for which less than 1% of the applied seeds were removed were considered 'not found'. It was assumed that this low proportion of seeds could have been lost due to wind, cracks in the soil, counting or weighing errors, or sampling errors. The proportion of patches 'found' was calculated per block. Seed removal rate was calculated for either all patches or only for the patches that were 'found'.

The only seed predator observed during the experiment was the harvester ant *M. barbarus*. However, we cannot exclude the possibility that some predation took place during the night. In that case, carabid beetles or rodents may have been active. However in the same area, Baraibar et al. (2009) found extremely low densities of granivorous carabids or rodents. So, the probability that seeds were taken by other animals than harvester ants was extremely low. Furthermore, no other ant species than *M. barbarus* were observed.

As an estimate of ant density, the number of ant nests was counted in all blocks, using a 10 m × 10 m grid, from 14 to 15 July. Nests were marked with spray-paint to prevent double counting. Nests were counted per block and transformed to nests per hectare. Nests were only included if ant activity was detected, to prevent the inclusion of

abandoned nests. Counting was therefore done from sunrise until late morning, because temperatures above 35 °C reduce ant activity (Azcárate et al. 2007).

Data analysis

Sampling efficacy

The sampling efficacy, E, of the various suction devices was calculated per block and per initial seed density as the percentage of the ratio between seeds recovered (Sr) and initial seeds applied (expressed in weight) (Si) in efficacy patches:

$$E = \left(\frac{Sr}{Si} \right) \times 100 \ \% \tag{2}$$

Generalized linear regression (GLM analysis, binomial distribution, logit link function, Genstat 11) was used to determine the effect of block, suction device (V_1, V_2 and D-Vac) and initial seed density and interactions, as independent variables, on the sampling efficacy in the efficacy patches using the proportion of recovered seeds as response variable. This information was used to correct seed retrieval from patches exposed to harvester ants.

Seed removal

The seed removal rate, R, was estimated per seed density and per block, as the proportion of removed seeds in exposed patches (Sr) relative to the initial seeds applied (in weight) (Si), corrected for E,

$$R = \frac{\left(Si - Sr * \dfrac{1}{E}\right)}{Si} \times 100 \ \%$$

(3)

A generalized linear mixed regression model (GLM analysis, binomial distribution, logit link function, Genstat 11) was used to explain the effect of block, initial seed density and interactions on the proportion of seeds removed. Preliminary analysis showed that one of the four blocks yielded a very different result from the other three blocks. Therefore, analyses were conducted for each block separately, and 'suction device' was entered again as an explanatory variable. Patches were included as a random factor and block, suction device and initial density as fixed factors. Only patches found by predators were included in the analysis (see in Results section).

Results

Apart from *A sativa* L., no others seeds were found in any of the control patches. This means that patches were free of *L. multiflorum* prior to seed application and therefore, the proportion of removed seeds in exposed patches, (*Sr*) in formula (2), did not have to be corrected for background seed density. Seeds of *A. sativa* were found in control patches but not in the exposed patches. It is likely that some *A. sativa* seeds had been present in the exposed patches too but that the presence of the applied *L. multiflorum* seeds resulted in enhanced foraging of *A. sativa* seeds. We assumed that the *A. sativa* seeds did not affect the seed removal rates of *L. multiflorum* seeds. Ant nest densities were 896, 468, 284 and 900 nests ha^{-1} in blocks A, B, C and D, respectively.

Table 3.1 Efficacy of suction devices (vacuum cleaners V_1 and V_2, and D-Vac) in blocks A, B, C and D using different initial seed densities 1 (1000); 2 (2000); 3 (5000); 4 (10000) and 5 (20000), (seeds m^{-2}) or not sampled (/).

Suction device	Density	Suction device	Density
Block A		**Block C**	
V_1	1, 2, 3, 4, 5	V_1	1, 2, 3, 5
V_2	/	V_2	2, 4, 5
D-Vac	1, 2, 3, 4, 5	D-Vac	1, 2, 4
Block B		**Block D**	
V_1	/	V_1	2, 3, 4, 5
V_2	1, 2, 3, 4, 5	V_2	1, 2, 4
D-Vac	1, 2, 3, 4, 5	D-Vac	2, 3, 5

Sampling efficacy

In general, the efficiency of the various machines at retrieving seeds after application of *L. multiflorum* seeds was high. The efficiency was highest (86%) for those patches that had been seeded with 20000 seeds m^{-2} and lowest (69%) for patches seeded with 10000 seeds m^{-2} (Fig. 3.1). The sampling efficacy was not influenced by the suction device used by block, or by the initial seed density (Table 3.3). The average percentage recovered (77% ± 0.28) was used as a correction factor (*E* in formula 1) in further calculations.

Table 3.2 Average percentage of seed removal of *L. multiflorum* seeds (N= number of samples; ± SE) by the harvester ant *Messor barbarus* for blocks A, B, C, and D, for suction devices V_1, V_2 and the D-vac, and for different initial seed density (seeds m^{-2}).

Initial density [seeds m^{-2}]	Suction device	% seed removal for 24 h											
		BLOCK A			BLOCK B			BLOCK C			BLOCK D		
		N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
1000	V_1	5	99.03	1.05	0	-	-	1	99.97	-*	4	99.99	<0.01
	V_2	0	-	-	8	99.92	0.08	5	99.95	0.04	3	100	0
	D	5	99.97	0.02	2	100	0	3	81.46	22.70	3	100	0
	Total	10	99.50	0.49	10	99.94	0.06	9	93.79	6.55	10	99.99	<0.01
2000	V_1	4	99.95	0.04	5	100	0	0	-	-	1	100	-*
	V_2	0	-	-	2	99.95	0.03	2	99.97	0.04	3	100	0
	D	6	99.99	0.01	3	100	0	8	76.57	16.36	6	100	0
	Total	10	99.97	0.02	10	99.99	<0.01	10	81.25	13.14	10	100	0
5000	V_1	4	100	0	2	100	0	0	-	-	6	100	0
	V_2	0	-	-	3	99.99	<0.01	3	99.98	0.01	0	-	-
	D	6	99.99	<0.01	5	100	0	6	99.98	<0.01	4	100	0
	Total	10	99.99	<0.01	10	99.99	<0.01	9	99.98	<0.01	10	100	0
10000	V_1	6	99.99	<0.01	3	100	0	0	-	-	0	-	-
	V_2	0	-	-	3	100	0	6	99.99	<0.01	5	100	0
	D	4	100	0	3	100	0	1	99.99	-	5	99.99	<0.01
	Total	10	99.99	<0.01	9	100	0	7	99.99	<0.01	10	99.99	<0.01
20000	V_1	4	99.99	<0.01	3	99.99	<0.01	0	-	-	1	-	-
	V_2	0	-	-	3	100		2	99.94	0.03	2	99.99	<0.01
	D	6	99.97	0.03	4	100	0	5	99.98	0.01	7	99.99	<0.01
	Total	10	99.98	0.01	10	99.99	<0.01	7	99.97	0.01	10	99.99	<0.01

applied to1 m² patches.

* single value.

Table 3.3 Effect of block (A, B, C or D), suction device (vacuum cleaners V_1 and V_2 or D-Vac) and initial seed density (g seeds m^{-2}) on the efficacy of the suction devices immediately after seed application. (Genstat GLM, binomial distribution, logit-link function).

Effect	d.f	Deviance	mean deviance	deviance ratio	*P*
block	3	1.04	0.35	0.43	0.75
suction device	2	0.53	0.27	0.33	0.75
initial density	4	0.80	0.20	0.25	0.89
block x initial density	12	1.10	0.09	0.11	0.99
block x suction device	5	0.64	0.13	0.16	0.96
suction device x initial density	8	0.44	0.05	0.07	1.00
block x suction device x initial density	3	0.16	0.05	0.07	0.97
residual	2	1.61	0.80		

Seed removal

The generalized linear mixed regression models showed that the initial seed density significantly influenced seed removal rates in all blocks, except block D. There was a residual effect caused by the suction device despite the initial correction (*E*; see above). Therefore, the factor suction device was retained in the mixed model. Interaction between initial seed density and suction device was significant in blocks A and D, but not in blocks B and C (Table 3.4).

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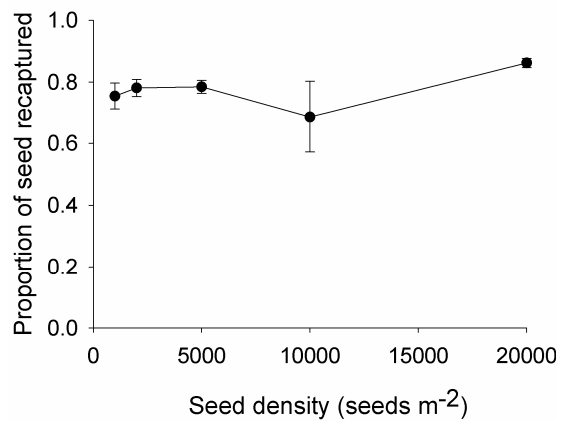
Fig 3.1 Proportion of seed recapture for suction devices V_1, V_2, and D-Vac averaged over all blocks as a function of seed density of *L. multiflorum*; seed density 1000, 2000, 5000, 10000 and 20000 m^{-2}. Bars represent standard errors.

Table 3.4. The effect of suction device (vacuum cleaners V_1 and V_2 or D-Vac) and initial seed density (g seeds m^{-2}) on the proportion of removed seed (Genstat GLM, binomial distribution, logit-link function).

Effect	d.f	deviance	mean deviance	deviance ratio	*P*
Block A					
suction device	1	0.06	0.06	15.93	<0.001
initial density	1	0.08	0.08	21.83	<0.001
initial density x suction device	1	0.04	0.04	12.11	0.001
residual	46	0.17	<0.01		
Block B					
suction device	2	0.01	<0.01	9.73	<0.001
initial density	1	<0.01	<0.01	8.37	0.006
initial density x suction device	2	<0.01	<0.01	2.44	0.099
residual	44	0.03	<0.01		
Block C					
suction device	2	2.98	1.49	5.62	0.008
initial density	1	3.18	3.18	11.98	0.001
initial density x suction device	1	0.03	0.03	0.12	0.73
residual	36	9.55	0.27		
Block D					
suction device	2	<0.01	<0.01	3.98	0.024
initial density	1	<0.01	<0.01	0.14	0.706
initial density x suction device	2	<0.01	<0.01	5.20	0.009
residual	44	<0.01	<0.01		

All patches at all densities were quick detected (17. 4 ± 1.6 min in block A, 15.8 ± 1.5 min in block B and 16.4 ± 1.5 min in block D) after seed application, and all applied seeds were collected (99.5 to 100%) within 24 h. In block C, ants discovered 39 patches within two hours after seed exposure which resulted in a final seed removal rate of 99.9% after 24 h (Table 3.2). Three patches were only found 21 hours after seed exposure, when we arrived to prepare for recovering the remaining seeds. Of those three patches, seed removal rates after 24 h were 44% (initial density 1000 seeds m^{-2}), 11% and 2% (initial seed density 2000 seeds m^{-2}). That is the reason why, in block C, average seed removal rates for densities 1000 seeds m^{-2} and 2000 seeds m^{-2} were only 94% and 81%,

respectively (Table 3.2). After 24 h, eight patches were still not discovered by seed predators. There was no relationship between initial seed density and whether or not a patch was found. Of the patches that were not found, one had been seeded with 1000, one with 5000 seeds m^{-2}, three with 10000, and three with 20000 seeds m^{-2}.

Discussion

A direct density-dependent response of harvester ants to increasing seed densities would be desirable to help reducing weed patch growth and persistence. We found that seed removal rate neither increase nor decrease as a function of weed seed density. The reason for the absence of a density dependent response was that removal rates by harvester ants were extremely high. Maybe a density dependent response would have been observed if much lower or much higher seed densities had been used. However, it is unlikely that densities much higher than those used in the experiment would naturally occur in the field. At lower densities, harvester ants do not form foraging trails and search for seeds individually (Wilby and Shachak 2000), so density dependent responses by harvester ants may only occur at densities lower than 1000 seeds m^{-2}.

Quick patch detection and high foraging rates were observed in the blocks A, B and D, which had the highest nest abundance (> 480 ha^{-1}). Because nest density was so high, there were always one or more nests within 1.6 m from the perimeter of a patch. Therefore, patch detection was quick (within 30 minutes). In block C, where nest density was lowest (284 nests ha^{-1}), low removal rates found in three patches could be due to a late patch encounter by ants, which allowed little time to remove seeds before the end of the experiment (24 hours). In addition, ants failed to discover 8 patches (16 %). There are

three possible reasons why patches were not or late detected. Firstly, within the 24 h that seeds were available, it is possible that the foraging ants were busy exploiting another part of their territory. Secondly, because of the lower nest density in block C the average distance between patch and nest entrance was 2.8 m instead of 1.6 m, resulting in a lower detection probability. And thirdly, at low nest densities, the nests may occur more aggregated, in particular if the colonies are small whereas the spatial distribution of nests at high densities tends to be more uniform. In the case of clumping of nests, the average distance between patches and nests will be larger than when nests are uniformly distributed. It is not clear why nest density was lower in block C than in the other blocks. The reasons could be related to unfavourable soil characteristics (Diaz 1991; Johnson 2000). The slope of around 1 m in this block could cause less favourable soil conditions and provoke a low(er) survival probability of founding queens and new colonies (Diaz 1991).

The extremely high removal rates in this study could have been the consequence of using a highly preferred weed species, like *L. multiflorum*. Had a less-preferred species, such as *B. diandrus* (Westerman et al. 2012) been used, harvester ants may have showed lower removal rates compared to *L. multiflorum*. Other harvest ant species have shown similarly low foraging activity towards patches with less-preferred species; however when the seed density of preferred species becomes low they may shift the foraging activity towards low preferred species (Pirk et al. 2009). Another reason could be the high seed densities offered to harvester ants that could induce the formation of pheromone trails and cause high removal rates. At lower densities, harvester ants search

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individually not forming trails (Wilby and Shachak 2000) and likely, with lower removal rates.

Extremely high seed removal rates regardless of the seed densities were related to high ant nest densities, which in turn might be related to the fact that these fields were managed without tillage. On the contrary, in conventionally tilled fields nest density is lower than 300 ha^{-1} (Baraibar et al. 2009; Baraibar et al. 2011). Harvester ants responded density independently to a range of weed seed densities. If harvester ants had responded inversely density dependent, patches of higher seed density would have persisted. Despite the fact that the harvester ants responded density independently to increasing seed densities, it is likely that weed patches have few possibilities to survive in fields that harbour large populations of harvester ants, because the seed predation rates were so extremely high. The only possibility for weed patches to persist is if they happen to be located in an area with fewer nests. To improve biological weed control in dryland fields in NE Spain, reasons for spatial variability in ant (nest) density should be investigated and constraints for nest establishment removed.

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CHAPTER 4

Effect of weed patch size on seed removal by harvester ants

Abstract

In dryland cereals in NE Spain the harvester ant, *Messor barbarus* L., is responsible for 46-100% of seed removal and contributes to weed control. For effective weed control, the harvester ants have to be able to find and exploit patches of seeds. However, patch size might influence the probability of being discovered and the exploitation rate, i.e. larger patch size facilitate patch encounter due to larger perimeters and higher area-to-perimeter ratio. Therefore larger patches are expected to be more exploited by predators. Here, we studied the influence of the size of weed patches on seed removal rates by harvester ants.

Seed patches varying in size from 0.25 to 9 m^2 were randomly created in three 50 x 50 m areas in a cereal field after harvest. After 24 h of seed exposure, the remaining seeds were collected and used to estimate seed removal rates. Harvester ant nests in each block were counted as an estimate of population density.

Seed removal rate was lowest in the smallest (78-94%) and highest in the largest patches (99-100%). Ants were unable to find seven small size patches. When patches were found, the exploitation rate was the same. One area harboured fewer ant nests in a part of its range; here one the largest patches was only partially harvested (73%), probably because it was discovered late, such that not all seeds had been harvested by the time of the evaluation.

Observed differences in seed removal rate were mainly caused by differential encounter rates of patches of different size and harvester ant density. Preserving or enhancing nest densities in dryland cereal fields could be a way to enhance weed seed predation.

Introduction

Seed mortality by seed predators (birds, rodents or insects) is a biological process that may limit weed population size in arable land (Westerman et al. 2008; Westerman et al. 2009). This could prevent the growth and spread of large weed populations or could cause their extinction. However, its success depends in part on predator ability to detect weed patches (Baraibar et al. 2012) and how they respond to the size of patches (Searle et al. 2006; Moenting and Morris 2006).

Weed populations have a patchy distribution with patches of varying size and density and areas with few or no plants and thus seeds (Mortensen et al. 1993; Cardina et al. 1995; Johnson et al. 1996). Weed patches may be stable over time and space, or they may shrink or expand (Dieleman and Mortensen 1999). Several factors influence weed patch dynamics, such as seed dispersal (Shirtliffe et al. 2002), seed persistence (Heijting et al. 2007) and competition between weeds (Blanco-Moreno et al. 2004). Furthermore, patch size and shape are shaped by human-aided dispersal of seeds, such as through tillage and harvest (Blanco-Moreno et al. 2004), crop competition (Webster et al. 2000), and herbicide treatment (Johnson et al. 1996).

Patch size may influence the probability of encounter and exploitation. Patch perimeter and area-to-perimeter ratio determine the probability of patch detection by predators (Orrock et al. 2003). The kind of response will depend on the type of predators and the scale at which they forage. Predators with a large action radius are more likely to encounter large patches. Nevertheless, they may fail to encounter or ignore small patches (Ritchie 1998). Those predators are able to recognize area size and the profitability often by their visual or olfactory cues (i.e. Hulme 1993). Birds forage in a large scale and their selectivity to patch size may differ between species (Moorcroft et al. 2002; Schlossberg

and King 2008). Rodents differ in the scale of foraging. Granivorous rodents have a relatively large action radius due to their high mobility, however they are found to forage preferentially towards small patches (Tew et al. 2000). Some rodents such as *Microtus* sp. are unable to determine area and use only the frequency of encounter with perimeter in small scale (Moentig and Morris 2006). Predators which forage at a relatively small scale perceive patches at this smaller scale and are more likely to encounter small patches (Ritchie 1998). Carabid beetles are much less mobile than rodents or birds, they usually move ten meters per day and they are more likely to select small weed patches (Zhang et al. 1997). Invertebrates show lower mobility than large-scale predators and they are less likely to recognize the area. Therefore, patch perimeter should determine their encounter rate rather than area.

Once a patch is encountered, predators decide to exploit it or to leave (Stephens and Krebs, 1986; Wellenreuther and Connell 2002). They may recognize the profitability of the patch instantaneously, moving quickly into, while other predators have to explore the patch quality during the exploitation (Schmidt and Brown 1996). Predators have to assume the handling time and energy that is necessary to spend exploiting the patch, i.e. for larger patches they need more handling time and effort due the higher total amount of prey (Wellenreuther and Connell 2002). Despite of higher time cost and effort, large scale predators will use large patch in order to spend less time in searching (Ritchie 1998). However, some predators may avoid exploiting larger patches i.e if there is possible predation risk for them during the exploitation. The availability of resources out of the patch may also determine the patch depletion (Brown 1988). Little is known how social insects could respond to different patch size.

In dryland fields in NE Spain, the main seed predator is the harvester ant *Messor barbarus* L. (Baraibar et al. 2009). This seed predator can cause up to 46-100 % losses of seeds of weed species (Westerman et al. 2012). Harvester ants usually forage in thirty meters action range (Azcárate and Peco 2003). *Messor barbarus* may encounter patches of low and higher profitability and change their foraging strategies according to patch quality. Quality can be defined as the patch area, seed amount, seed species, etc. (López et al. 1993; Detrain et al. 2000; Heredia and Detrain 2005). High quality patches induce formation of the trials with higher number of brunches and higher patch consumption compared to low quality patches (López et al. 1993). However low quality patches promote trails with smaller number of branches that slows down patch exploitation (López et al. 1993). Ants select more profitable patches from other resources based on the higher amount of laid pheromones on trails, higher frequency of ants (Detrain et al. 2000), the shorter time of seed retrieval from the source to the nest (Heredia and Detrain 2005) and the quantity of pheromones per worker (Heredia and Detrain 2000). Therefore, we predicted that frequency of encounter with patch perimeter should influence their response to the size of patch.

In our study, we were interested how different patch size could influence seed removal rate by harvester ants. In order to ensure the response to the patch perimeter, we applied the patches of four sizes with the equal total perimeter per each size. The same seed density was used to exclude its effect on the response. We assumed that larger patches should increase the probability to be encountered due the higher patch perimeter and area-to-perimeter ratio. Consequently, higher exploitation rate is expected in larger patches.

Material and Methods

A trial was conducted in a commercial, no-till cereal field, shortly after crop harvest, in Villanova de Bellpuig (41° 35′ 25.76″ N, 0° 58′ 36.28″ E, sandy clay soil) in NE Spain in 2010. An experimental area (150 × 50 m) was divided into three blocks (A, B and C) of 50 × 50 m each, at least 20 m from the field margin. In each block, 38 square areas (called 'patches') were located randomly. Five patches per block (1 m^2; 1 m × 1 m) were used to obtain information on the density of seeds naturally available on the soil surface before seed application. Three patches per block (1 m^2) were used to test the efficiency of the machinery used to retrieve seeds (efficiency patches). The remaining 30 patches per block were used to estimate seed removal by predators in response to patch size (exposed patches).

The exposed patches were available in four different sizes; 16 patches of the smallest size (size 1; 0.25 m^2; 0.5 m × 0.5 m); eight patches of medium-small size (size 2; 1 m^2; 1 m × 1 m); four patches of medium-large size (size 3; 3 m^2; 1.73 m × 1.73 m) and two large patches (size 4; 9 m^2; 3 m x 3 m). The patches were randomly distributed within a block, with a minimum distance of 1 m between patches. The number of patches per size class was such that the cumulative perimeter of all patches was identical between size classes (see Discussion section). Straw was removed from the patch areas by raking and sweeping. The outline of each patch was carved in the soil surface with a knife and by coloured stakes in the corners. Experiments were conducted one month after harvest, as three replications in time: 10 August in block A, 16 August in B, and 17 August in C. After one day of seed exposure (24 h), the remaining seeds were collected.

Avena sativa L. seeds (Semillas Batlle, Bell-Lloc, Spain) were used here because they should be readily taken by harvester ants (Lopez et al. 1993), and because they could easily be distinguished from straw and soil, which facilitated seed recovery and counting. The same seed density, namely 2000 seeds m^{-2}, determined by weighing, using a seed weight of 73 ± 0.4 g per 2000 seeds, was applied in all patches (Table 4.1). To obtain a uniform seed weight, seeds were dried in the oven at 40 ˚C for 4 hours, both before weighing prior to application and after retrieval prior to re-weighing. Seeds were uniformly applied by hand on the surface during the early morning hours (from 7:00 until 7:30 am). This was done while wearing gloves, as ants may avoid seeds that have been handled by humans.

Twenty four hours after seed application, seeds were retrieved using a D-Vac (Vortis; Burkard manufacturing Co. Ltd., Rickmansworth) for two minutes per square meter. In the case of patches of sizes 1 and 2, the entire area was vacuum cleaned. In patches of size 3, two sub-areas of 1 m^2 were vacuum cleaned; in patches of size 4, three sub-areas of 1 m^2 were vacuum cleaned. All material on the soil surface was collected in a paper bag. The samples consisted of seeds, dry soil particles and remnants of plant debris. After retrieval, samples were dried, sieved and cleaned from the straw, and weighed to estimate the number of seed retrieved.

To assess the density of seeds naturally available on the soil surface before applying seeds of *A. sativa*, five control patches of 1 m^2 in each block were sampled one day before seed application. Soil surface samples were collected in paper bags and processed as described above.

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The efficiency of the D-vac at retrieving the applied seeds was determined in three randomly selected patches per block. Seeds may not have been retrieved because they may have fallen in cracks and crevices, such as found around the base of cereal stubble. Seeds were manually applied on the soil surface one hour before seed application in the exposed patches. Seed were retrieved immediately after application to avoid seed removal by ants. Soil surface samples were collected in paper bags and processed as described above.

Ant activity is influenced by soil temperature; they are most active when the soil temperature is between 15 and 35 °C (Azcárate et al. 2007). Therefore, average hourly temperatures were obtained from a weather station located in Tornabous (46°17′40″ N, 33°73′16″ E), 10 km from the site of experimentation (Generalitat de Catalunya, 2012).

Ant nest density was determined by counting all nests in each block, using a 10 m × 10 m grid, on 10 August. Nests were identified by their entrances but only included if ant activity was detected, as to prevent counting of abandoned nests. All nests were marked with spray-paint to prevent double counting. Nest number was transformed to nests per hectare.

Data analysis

Sampling efficiency

The sampling efficiency, E, of the D-Vac was calculated as the ratio between the weight of the seeds recovered (S_r) and the initial seed weight (S_i): applied per square meter.

$$E = \left(\frac{S_r}{S_i} \right) \quad\quad\quad (4)$$

The average sampling efficiency across all efficiency patches and blocks ($\overline{E}$) was used to correct seed retrieval when exposed to harvester ants (see below).

Patch exploitation threshold

The lowest value found for the efficiency of the D-vac across patches and blocks, X, was used as a conservative estimate of Y ($=1$-X), the threshold value beyond which patches were assumed to have been found and exploited by ants. Any patch from which the proportion of seeds lost was higher than Y was assumed to have been found and exploited by ants.

Seed removal

The seed removal rate, R, was estimated as the difference between initial and recovered seed weight, corrected for E, (S_i-S_r) relative to the the initial seed weight (Si),

$$R = \frac{\left(S_i - S_r * \dfrac{1}{E}\right)}{S_i} \times 100\,\% \qquad (5)$$

A linear mixed regression model (Binomial distribution, logit link function, Genstat 11) was used to explain the effect of block, patch and size of the patch on the proportion of seeds removed. Patches were included as random factor and block and patch size as fixed factors. Because the factor block was significant, posterior analyses were performed for each block separately with patch as random factor and size as fixed factor.

Results

Control patches contained no seeds, except for 30 ± 5.4, 22 ± 6.3, and 85 ± 8.3 *Hordeum vulgare* L. seeds m^{-2} in blocks A, B, and C, respectively. This means that seeds of *Avena sativa* were not present prior to seed application. No ant species other than the harvester ant *M. barbarus* were observed. Previous studies found extremely low densities of granivorous carabids or rodents in the study area (Baraibar et al. 2009). So, it was assumed that the main seed predator in the experiment was the harvester ant *M. barbarus*.

Table 4.1 Patch characteristics of size, shape, the side length of the patch. Seed density of *A. sativa* (seeds m^{-2}), number of patches per block, patch perimeter (m) and area (m^2) per each size and block. Total seed number per each size and block and the area/perimeter ratio (A/P).

Patch size class	Shape	Side length (m)	Seed density (seeds m^{-2})	Nº of patches per block	Perimeter (m)		Area (m^2)		No Seeds		A/P ratio
					patch	block	patch	block	Patch	block	
1	rectangular	0.5	2000	16	2	32	0.25	4	500	8000	0.1
2	rectangular	1	2000	8	4	32	1	8	2000	16000	0.3
3	rectangular	1.73	2000	4	7	28	3	12	6000	24000	0.4
4	rectangular	3	2000	2	12	24	9	18	18000	36000	0.8

The densities of ant nests were 416, 436 and 428 nests ha^{-1} in blocks A, B and C, respectively (Table 4.3). Because ant nest abundance was similar in all blocks it was assumed not to affect seed removal rates in patches of different size or in blocks.

Table 4.2 The effect of patch size: 1 (0.25 m^2), 2 (1 m^2), 3 (3 m^2) and 4 (9 m^2) in block A, B and C on the proportion of removed seeds (Genstat GLM, binomial distribution, logit-link function).

Effect	d.f	deviance	mean deviance	deviance ratio	*P*
Block A					
size	3	128.36	42.79	7.35	<0.001
residual	34	197.85	5.82		
Block B					
size	3	185.08	61.69	2.67	0.063
residual	34	786.55	23.13		
Block C					
size	3	59.92	19.973	5.85	0.002
residual	34	116.16	3.416		

Because the blocks were seeded sequentially, temperature differed between blocks during the night and the late morning. The average temperature during the 24 h exposition was 25.3 °C in block A, 20.4 °C in block B and 24.1 °C block C (Fig 4.1).
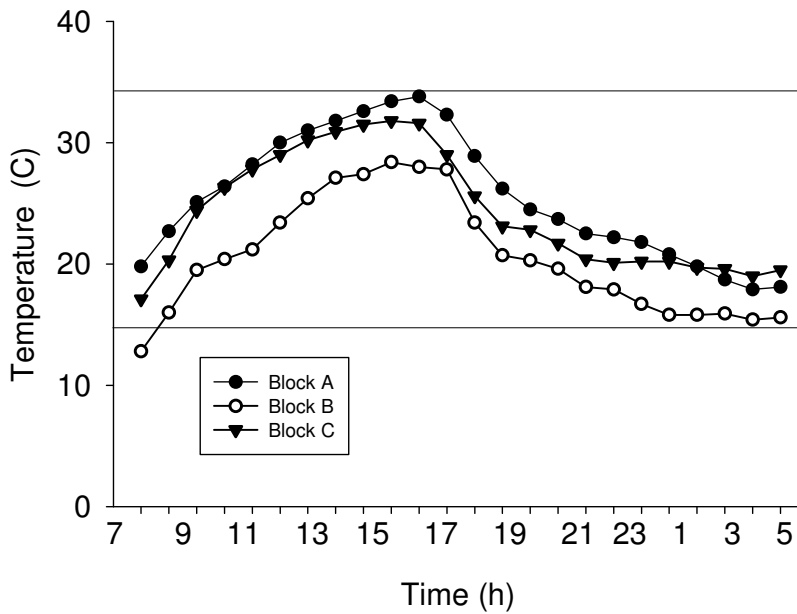
Fig 4.1 Average Temperature for 24 hours of the experiment in block A (●), B (○) and C (▼).

Table 4.3 Nest density (ha^{-1}) for each nest size (1, 2, 3, 4 and 5) in blocks A, B and C.

Block	Nest size	Nest density (ha^{-1})
A	1	164
	2	148
	3	96
	4	8
	5	0
B	1	216
	2	144
	3	68
	4	8
	5	0
C	1	276
	2	92
	3	52
	4	8
	5	0

Sampling efficiency

The average sampling efficiency ($\overline{E}$) of the D-Vac was 93.8% (range from 91.3-97.7%), which was used to correct further calculations to estimate seed removal. The lowest seed retrieval estimated for efficiency patches was 91.3%. So, the threshold seed removal rate (Y) was 8.7%. Patches with seed removal rate lower than 8.7% were considered as not detected by harvester ants.

Seed removal

Patch exploitation was close to 100% in most of the exposed patches. Two, four and one patches were not found by harvester ants in blocks A, B and C, respectively (Table 4.4). These patches were also included in the analysis. The size of the patch significantly influenced the seed removal rate in block A and C (P<0.01) but not in block B (P=0.063) (Table 4.2). In block A, harvester ants removed 87% from the smallest patches (size 1; 0.25 m^2), and > 99% from patches of the size 2, 3 and 4. In block B, harvester ants removed 78% from the smallest patches, 82% from patches of the size 2, 100% from patches of size 3, and 86% from patches of size 4. In block C, harvester ants removed 94% from the smallest patches (0.25 m^2) and > 99% from patches of size 2, 3 and 4 (Fig 4.2).
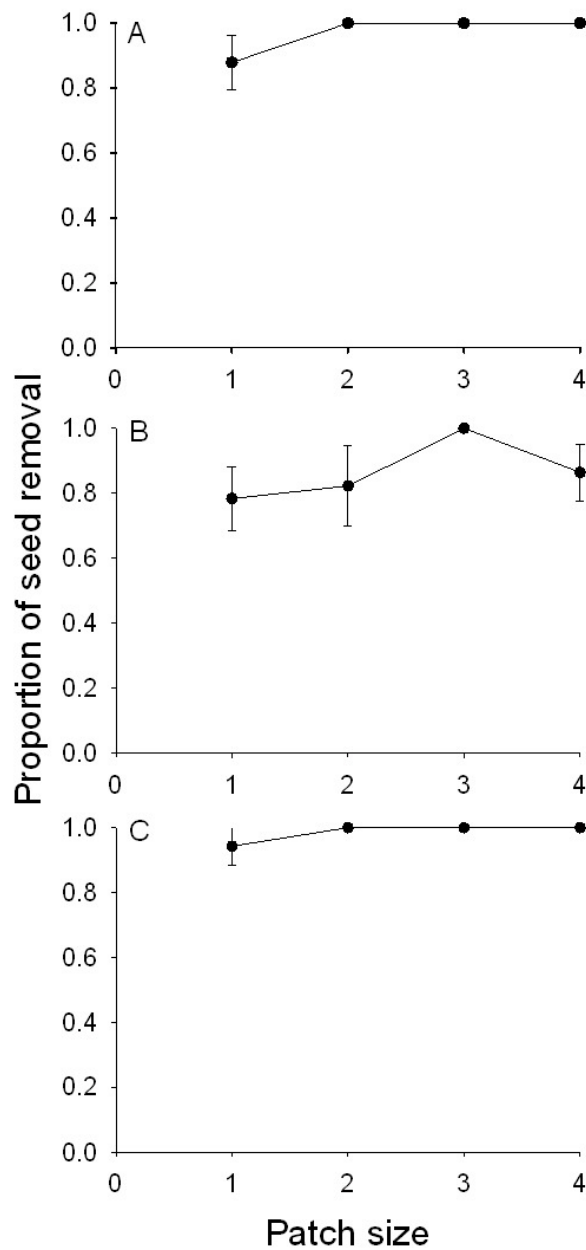
Fig 4.2 Proportion of removed seeds (day^{-1}) of *Avena sativa* as a function of patch size 1 (0.25 m^2), 2 (1 m^2), 3 (3 m^2) and 4 (9 m^2) in blocks A, B and C. Bars represent standard errors.

In block B, patch size did not significantly influence seed removal rate, because of the low removal rate in the largest patch size (Fig 4.2). The seed removal rate in the two

largest patches was 100 and 73%. We found that a single small nest within the latter patch was responsible for exploitation and no other nests were observed in a radius of 10 m (Torra, personal observation). Therefore, this particular patch was located in an area that happened to have a low density (Table 4.3) of harvester ant workers. Thus, the lack of overlap between patch and ant nest distribution was likely to be responsible.

Table 4.4 Number of not found patches by harvester ants in block A, B and C.

Block	Patch area (m^2)	Number of not found patches
A	0.25	2
	1	0
	3	0
	9	0
B	0.25	3
	1	1
	3	0
	9	0
C	0.25	1
	1	0
	3	0
	9	0

Discussion

In dryland cereal fields, the removal rate of oat seeds by harvester ants *M. barbarus* was the lowest for the smallest patches and increased with patch size (Fig 4.2). The reason for the decreasing removal rate with decreasing patch size was the lower probability of encountering the smaller patches; seven of the smallest patches were not found (Table 4.4). A small patch has a shorter perimeter and a smaller A/P ratio compared to a larger patch (Table 4.1). In this research, the total perimeter per patch size was kept constant.

Unlike, in other studies the same area was used to ensure the response of individual predators, i.e. birds are able to recognize the size of area. Rodents may increase mobility and residence time towards larger areas (Orrock et al. 2003). We did not have any data available for colonial insects and we assumed that ants should encounter the patch during random searching similar to other invertebrates. So, they are likely to respond to the patch perimeter due the probability of encounter, but not to the patch area. For this reason, we decided to keep constant the total perimeter per each of patch size (see Material and Methods).

However, other factors besides patch area and perimeter could contribute to explaining the results. Three of the small patches that were not exploited by ants were close to patches of size 3 and 4. It is likely that these large patches, containing 6000 and 18000 seeds, respectively, could have diverted the ants away from the smaller and towards the more profitable resources (Lopez et al. 1993; Detrain et al. 2000). Furthermore, four of the smallest patches that were not found by the ants were located in block B, which was sampled during the period with the lowest average temperature (Fig 4.1). Given the relatively low activity, the harvester ants may simply have had not enough time to found and exploit all patches.

Compared to other invertebrates that select smaller weed patches (Westerman et al. 2008; Baraibar et al. 2012), harvester ants may forage in a scale up to 30 meters (Azcarate and Peco, 2003). Furthermore, considering the short period of time that seeds were exposed to ants (24 h) and high removal rates, it is fair to say that harvester ants are more effective seed predators than rodents or other invertebrates studied in arable fields.

Seed removal rates by harvester ants were found to be positively related to patch size; when a patch was found, seed removal was almost always close to 100%. Therefore, harvester ants are expected to be able to regulate weeds successfully in patches of both large and small size. Ants prevent seeds from entering the seed bank, thus slowly exhausting the seed bank (Forcella 2003). Carry-over of viable *seeds* in the soil from previous years could buffer the effects of *weed* control by ants. For species with transient seeds, they may be able to eliminate patches because no form long term persistent seed banks are found (Baskin and Baskin 1998). Preserving of harvester ant nest density could enhance weed seed predation. Harvester ants could help to reduce the number of applications and amount of herbicides applied in dryland cereal fields.

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CHAPTER 5

The spatial distribution of nests of harvester ants (*Messor barbarus* L) in dryland cereals

Abstract

The harvester ant *Messor barbarus* L. is responsible for great losses of weed seeds in rain-fed area in NE Spain. The probability that a weed seed will escape predation will be lower for a field in which nests are regularly distributed than in a field where nests are more aggregated. Spatial distribution of *M. barbarus* nests and their interactions were studied in a rain-fed post-harvest oats field in order to assess the probability that weed seeds may escape predation due to unequal foraging intensities within a field. The objectives were to identify the degree of clustering as a function of spatial scale and ant nest density and to investigate the effect of nest size on spatial distribution, using second order spatial statistics based on Ripley's K function.

An experimental area was selected (150 × 50 m), at least 20 m from the field margin. The location of nests was geo-referenced using a high-precision GPS, and its size given according to a subjective scale ranging from 1 (small) to 5 (large). For each dataset, it was assessed the fit of global Strauss, multi-type Strauss and multi-type/hard core Strauss models, aiming to test for the presence of interactions between nests.

The nests arrangement was non-random. In 2009, the distribution of young nests varied from over-dispersed to more uniform distribution. Unlike, in 2010 all nest classes showed stable under-dispersion. The nest density was high with decline from 1652 to 1280 nests ha^{-1}, in 2009 and 2010 respectively and the reason was unknown. However, weed seeds have a low chance to escape predation, because nest density was really high and most nests were relatively regularly distributed. The high nest densities should be preserved to ensure the under-dispersion and therefore more equal exploitation by harvester ants over the field.

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Introduction

The harvester ant *Messor barbarus* L. is a common granivore in NE Spain. Inside rain fed arable fields, it is by far the most dominant granivore and responsible for formidable losses of weed seeds. Depending on the weed species, 46-100% of the newly produced weed seeds can be taken by the harvester ants (Westerman et al. 2012), thus contributing significantly to weed control. Seed predation risk can vary considerably among and within fields (Baraibar et al. 2009; Baraibar et al. 2011b). Differences in the spatial distribution of nests could be one of the factors responsible for the observed variability (Díaz 1992; Azcárate and Peco 2003; Baraibar et al. 2011b). The probability that an arbitrary seed will escape being detected will be lower for a field in which nests are regularly distributed than in a field where nests occur in clumps. In the latter case, some areas may be searched more intensely than others, as foraging intensity declines exponentially with distance from the nest (Crist and Macmahon 1992; Mull and MacMahon 1997; Azcárate and Peco 2003).

Summarizing 160 studies involving 136 ant species, (Levings and Traniello 1981) concluded that in the majority of the cases, nests were either regularly distributed (58), or randomly tending to regularly (76). However, nests were also found to be randomly distributed (12 cases), randomly tending to clumped (4 cases), clumped (2 cases), or distributed as were the host plants (8 cases). A simulation study showed that regularity in spatial arrangements of ant nests increased with 1) increasing mortality of founding queens and young colonies that settle near established nests, and 2) increasing nest density (density-dependent spacing) (Ryti and Case 1992). Competition between

established colonies, which affects colony survival and reproductive output, was less likely to result in a regular distribution of nests (Ryti and Case 1992).

Older colonies are expected to be more regularly spaced than young colonies. Once a year, mature colonies produce winged, reproductive males and females that are released after the first autumn rains. The location where the mated queen lands, is either randomly distributed in space or clumped (Ryti and Case 1988; Grohmann et al. 2010), but the probability of successfully nest initiation is strongly influenced by the proximity to long-established colonies (Hölldobler 1981; Ryti and Case 1988; Grohmann et al. 2010). Workers of larger colonies harass and kill invading founding queens and destroy smaller colonies (Baroni Urbani, 1968; Hölldobler 1981). Workers of younger colonies are more tolerant (Grohmann et al. 2010). Estimates of the mortality rate of founding queens and first year colonies vary between 90% (Ryti and Case 1988) and > 99% (Gordon and Kulig 1996). Survival probabilities of colonies remain low until they are about two years old (Johnson 2001); older colonies have higher survival chances. Older colonies managed to survive the selective sieve of competition, resulting in a more regular spatial distribution. As a consequence, differently sized colonies may have different distribution patterns (Korb and Linsenmair 2001).

It is predicted that intraspecific nest spacing is correlated with population density (Ryti and Case 1992). At population densities well below the carrying capacity of the area, there will be sufficient free space outside the territories of established colonies for foundresses to settle. The distribution of nests will, therefore, reflect the original landing sites of the queens, which will be either random or clumped. With increasing population density, the availability of space will become more and more limiting, resulting in

increased competition for space, leading to a more regular distribution of nests. The carrying capacity, and thus the maximum population density, in turn, will depend on the resources available in the area. However, the above prediction could not confirmed by experimental results (Schooley and Wiens 2003).

The spatial distribution of nests also depends on the scale of observation. The distribution of colonies is frequently regular or random at a fine spatial scale, but clumped at a larger spatial scale (Crist and Wiens 1996; Schooley and Wiens 2003; Folgarait et al. 2007). The spatial distribution of ants is, furthermore, influenced by external factors, in particular those that influence the survival chances of founding queens and young colonies, such as soil properties (Wiernasz and Cole 1995; Crist 1998; Enzmann and Nonacs 2010), topography (Crist and Wiens 1996; Kilpeläinen et al. 2008), microclimate (Kilpeläinen et al. 2008; Brown et al. 2012), vegetation (Bernstein and Gobbel 1979; Diaz 1991; Santini et al. 2011), land use, level of disturbance and field management (Diaz 1991; Serrano et al. 1993; Crist and Wiens 1996; Folgarait et al. 2007; Baraibar et al. 2009). For example, soil strength, texture and soil moisture content of the top soil determine the ease with which ants can tunnel the soil and construct chambers (Wiernasz and Cole 1995; Boulton et al. 2005; Enzmann and Nonacs 2010). Altitude, slope and orientation influence the number of hours that the ant nest is exposed to sunshine, which in turn influences ant activity and brood development (Crist and Williams 1999; Wang et al. 2001; Azcárate et al. 2007). Because these factors usually vary over large spatial scales, they manifest themselves as large-scale trends.

In this study, we investigated the spatial distribution of *M. barbarus* in a rain-fed post-harvest oats field in NE Spain, in order to assess the probability that weed seeds may

escape predation due to unequal foraging intensities within a field. The location of nests was geo-referenced using a high-precision GPS. The size of each nest was determined based on a subjective scale ranging from 1 (small) to 5 (large) (Baraibar et al. 2011b). The objectives were 1) to identify the degree of clustering as a function of spatial scale and ant nest density and 2) to investigate the effect of nest size on spatial distribution, using second order spatial statistics based on Ripley's K function.

Material and methods

The location of nests of *M. barbarus* was determined in a no-till cereal field, shortly after crop harvest, in Villanova de Bellpuig (41° 35′ 25.76″ N, 0° 58′ 36.28″ E, sandy clay soil) in NE Spain in 2009 and 2010. An experimental area was selected (150 × 50 m), at least 20 m from the field margin. The corners and perimeter of the selected area (every 50 m) were marked with a GPS with sub-metric precision (Trimble® GeoXHTM hand-held, GeoExplorer® 2005) that enabled to reinstall the experiment in the same area in 2010. To facilitate geo-referencing and counting of nests, the area was subdivided into 45, 10 × 10 m squares. Nests were marked with spray-paint andlabelled with colour stakes to prevent double counting. Nests were identified by their entrances, but only included if ant activity was detected, as to prevent counting of abandoned nests. The coordinates of each nest were recorded, during the period from sunrise at 7:00 until 12:00when temperatures allow ant activity (Azcárate et al. 2007). Geo-referencing was done on 14 July, 22 July and 4 August in 2009; and on 10 August, 16 August, and 17 August in 2010. Nest size, *S*, was estimated using a subjective scale from smallest (1) to largest (5) with regard to

surface area occupied by the colony, worker size and the number of active ants as described by (Baraibar et al. 2011b).

Methods for spatial pattern analysis

The choice of nest categories included into spatial analysis was based on the sample size and nest traits. Only sufficiently large samples could be analyzed, therefore the nests were grouped into new size classes. Categories 1 and 2 were regrouped into a new class S_1 and categories 3, 4 and 5 into a new class S_2. The reason was that class 4 and 5 had few nests (32 and four, respectively), and could yield meaningful results due to the small sample size. Furthermore, categories 1 and 2 were nests with only one entrance, while nests belonging to category 3 had more than one entrance (Baraibar et al. 2011a). As said, the implicit assumption is that nest size is related to age, larger colonies are older. The number of entrances is an indirect estimation of colony size, thus, more entrances could mean that the nest is older. For this reason, Categories 1 and 2 were joined as they were supposed to be of similar age (no more than two years), while categories 3, 4 and 5 were supposed to be older nests, i.e. three years or more.

Ripley's K function was used as a summary statistic to quantify the spatial structure of uni- and bivariate patterns, i.e. patterns of the same class (S_{11} and S_{22}), and patterns of two different size classes (S_{12} and S_{21}). Ripley's $S_{11}(r)$ function for univariate patterns of a given nest of size 1 can be defined such that $\lambda S_{11}(r)$ equals the expected number of additional nests of size 1 within a distance r of a typical nest of size 1. λ is the intensity of the process, i.e. the expected number of nests per unit area. The estimate of $S_{11}(r)$ derived from a spatial point pattern can be used in exploratory data analysis and

formal inference about the pattern (Cressie 1991; Diggle 1983; Ripley 1977, 1988). In exploratory analyses, the estimate of S is a useful statistic summarising aspects of inter-point "dependence" and "clustering". For inferential purposes, the estimate of S is usually compared to the true value of S for a completely random (Poisson) point process, which is $S(r)=\pi r^2$. Deviations between the empirical and theoretical S curves may suggest spatial clustering or spatial regularity. The same properties apply to the functions $S_{12}(r)$ or its reciprocal version $S_{21}(r)$ which describe the expected number of nests of size 2 around a typical random nest of size 1, or vice versa.

To account for possible first-order effects (trends) in the global density of nests resulting from larger-scale heterogeneity of the habitat, the heterogeneous Poisson process was used as null model. The inclusion of spatial trends in the null model allows the assessment of potential interactions among nests (second order effects), which may occur at scales smaller than the processes determining the trend. The occurrence of any nest in a heterogeneous Poisson point process is independent from that of others, but nests are distributed according to an intensity function $\lambda(x,y)$ which varies with location (x,y). A non-parametric kernel estimate of the intensity function based on the Gaussian kernel with a bandwidth of h = 10 m was used (Lan et al. 2012). In some cases, the nature of the data and the strength of trends in the observed pattern make decisions with regards to the degree of smoothing that is required easy. In other cases, decisions may be difficult and open to debate and interpretation (Wiegand and Moloney 2004). We have opted for an intermediate degree of smoothing, which preserves enough detail of the original data but does not strongly follow the distribution of individual nests.

To assess second order effects, different classes of Gibbs models of inter-point interactions were fit according to Baddeley and Turner (2006). For each dataset, the fit of global Strauss, multi-type Strauss and multi-type/hard core Strauss models were assessed, while testing for the presence of interactions between nests. The global Strauss process is intended to capture inter-nest repulsive interactions that do not depend on nest size, and therefore it is of the same range and strength regardless of nest size. Multi-type Strauss process models inter-nest interactions, but allows for different radii and strength of interactions depending on the nest size. Multi-type/hard core Strauss process is a further modification, which is intended to "decompose" the interaction between nests into two different components: one "hard core" radius, within which there cannot be any other nest around, and one interaction radius within which the probability of finding further nests is reduced. Profiled pseudo-likelihood was used to estimate the interaction radii (*ir*) of the Strauss models (irregular parameters), while the hard-core radii (*hr*) were replaced by their maximum likelihood estimates, the minimum distance between nests of a given size (Baddeley and Turner 2006). The significance of the models was tested by means of Monte Carlo methods against 249 simulations of the null model (yielding a minimum p-value of 0.004), the heterogeneous Poisson process (Baddeley and Turner 2006); the improvement of the model was measured and tested by means of the difference in log-pseudolikelihoods (Δlog(*pLik*) hereinafter) and the Akaike's Information Criterion (AIC) between the null and the alternative model (Table 5.1).

Table 5.1 Akaike Information Criterion and Log-pseudo-likelihoods of the null and the alternative models for 2009 and 2010.

Spatial model	AIC	log(*pseudo-Likelihood*)
2009		
Homogeneous Poisson	3570.49	-1783.24
Inhomogeneous Poisson	2499.27	-1243.63
Inhomogeneous MultiStrauss	2471.74	-1226.59
Inhomogeneous MultiStrauss-Hard core	2443.21	-1212.6
2010		
Homogeneous Poisson	2967.71	-1481.86
Inhomogeneous Poisson	2045.5	-1016.75
Inhomogeneous Strauss	2014.49	-1000.25
Inhomogeneous MultiStrauss	2008.92	-995.46
Inhomogeneous MultiStrauss-Hard core	1999.95	-990.97

To improve readability and interpretation of results, the L function for a spatial point pattern (Besag's transformation of Ripley's K-function) was used throughout. This function is a transformation of Ripley's K-function:

$$L(r) = \sqrt{K(r)/\pi}$$

This transformation stabilizes its variance, and has the desirable property of having an expected value equal to the radius r. The graphical representations take advantage of this fact, and the function is represented always as $L(r)$-r, so it has an expected value of zero. Positive deviations at a given radius r indicate an excess of ant nests from the expectation in a circle of radius r centred around a typical random nest; in other words, nests appear clustered. Similarly, negative deviations indicate a smaller number of nests from what would be expected by chance. In other words, nests appear segregated.

The fit of the models to the observed L-function was assessed graphically. Simultaneous critical envelopes were computed, using Monte Carlo methods. For a confidence level of α=0.05, these critical envelopes are obtained by first determining the expected value of the L-function, and then the 95th percentile of the maximum difference between the expected value and the L-function for the simulated data within a range of *r*. The confidence intervals are built simultaneously for all distances *r* from the expected value for the summary function plus and minus the 95th percentile of the differences. If the observed function lies outside the envelope obtained at any value of *r*, the null hypothesis (the agreement to a specified model) can be rejected. This Monte Carlo test has an exact significance level $\alpha = rank_{observed\ data} / (1 + number\ of\ simulations)$.

To avoid edge effects, a border correction (d=5) was applied in all analyses. Although more complicated forms of edge correction are available and sometimes preferred, they add a significant computational burden to the model fitting and methods of significance testing. The analyses were performed separately for 2009 and 2010 in each model.

A series of models was used to describe the spatial distribution of nests of harvester ants after the large-scale trend was removed. Models differed in the degree of computational complexity, assumptions and randomness, description of spatial distribution of nests and inter-nest interactions (independent or dependent of the nest size), hard-core distance and the interaction distance between nest size classes. Models were applied in increasing order of fit to the observed pattern.

First, Homogenous Poisson (Fig 5.2) model was used. This model showed strong departures from random distribution at all scales therefore Inhomogenous Poisson (Fig

5.3) model had to be used. The inhomogenous Poisson model included a non-parametric trend because there were no significant covariates that could explain the spatial trend (elevation at the field, distance from the edge, x and y coordinates) (Wiegand and Moloney 2004). This model did not fit (Table 5.1), because the interaction between nests caused departures from the expected trend. The Inhomogenous common Strauss model common to all nest sizes (Fig 5.4; 5.5) was used as a simple model of dependence between points (Baddeley and Turner 2006). However, the common Strauss model (Fig 5.5) fitted reasonably the distribution of nests and their interaction independent of the size. This model did not fit properly for all pair-wise interactions (Table 5.1). Multi-type Strauss (Fig 5.6) was used in order to explain spatial distribution and nest interactions with the nest size included. This model fitted better than previous models (Table 5.1), but there were indications of strong repulsion effects at short distances (interaction parameters $\hat{\gamma} = 0$). In order to ensure better fit Multi-type Strauss hard-core (Fig 5.7) was used. This model explained 1) the hard-core distance, the minimal distances where it is never expected the neighbor nest and 2) the interaction distance with reduced probability of presence of another nest. In this study, of the six models tested, Multi-type Strauss hard-core model fitted all criterions; randomness, hard-core distance, spatial distribution of nests, the inter-nest interactions dependent of the nest size. This model was favored over model Multi-type Strauss because it included variable hard-core distances.

Results

The distribution of nests differed between nest size classes in both years (Fig 5.1 A,B). There were areas with lower (blue colour) and higher densities (yellow/salmon colour).

Nest density (nests ha $^{-1}$) was higher in 2009 than in 2010. In 2009, there were 1336 nests ha $^{-1}$ of class 1, and 316 nests ha $^{-1}$ of class 2. In 2010, there were 1040 nests ha $^{-1}$ of class 1 and 240 nests ha $^{-1}$ of class
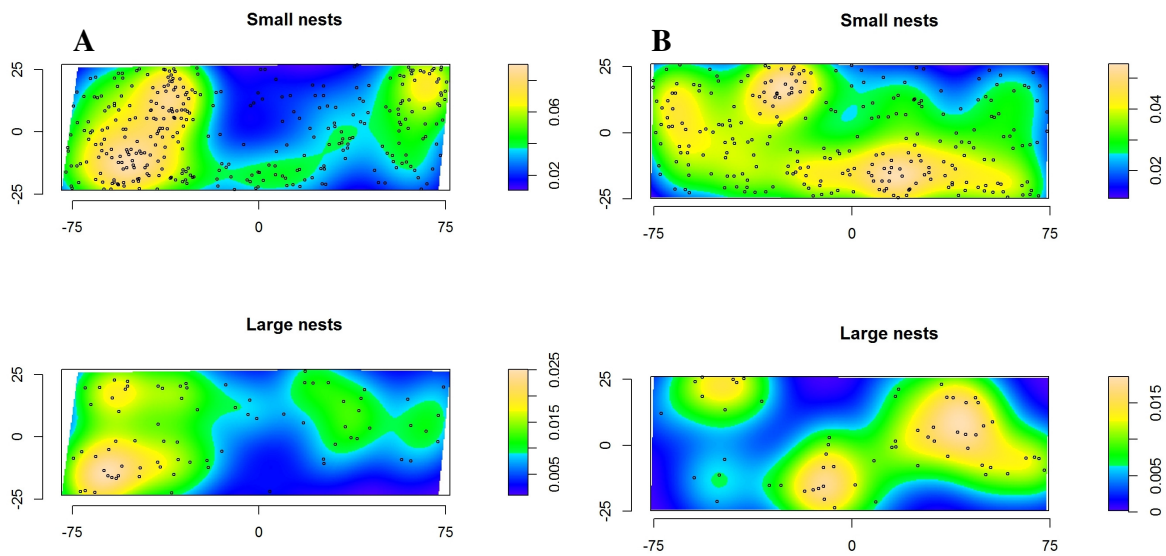

Fig 5.1 Maps of *M. barbarus* nest locations for small, S_1, and large nests, S_2, in 2009 (A) and 2010 (B). Nest density is related to colour, with blue referring to low densities and yellow/salmon to high densities. Note that the colour scales differ between years.
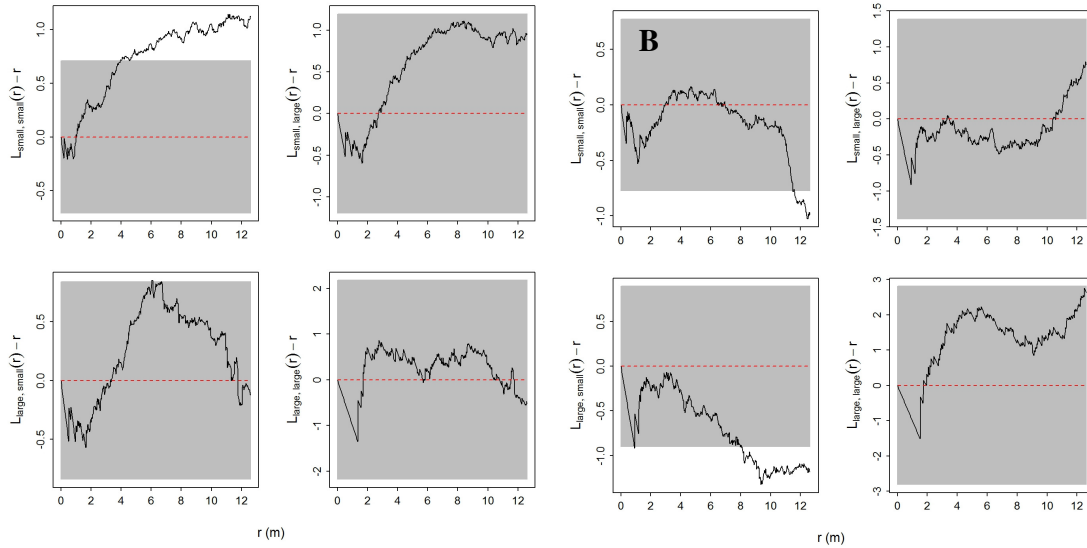
Fig 5.2 Estimated L-function for each pair of nest sizes, as a function of *r* in 2009 (A) and 2010 (B); within plots A and B, the left top pane shows the spatial distribution of nests size class S_1, the right top pane the arrangement of nests of size class S_1 around class S_2, the left bottom pane the arrangement of nests of size class S_2 around class S_1, and the right bottom pane the spatial spatial distribution of nest size class S_2. The red dashed line represents the expected value of the L-function under the homogeneous Poisson process, while the grey band represents the simultaneous 95 % critical envelopes.

In 2009, there was a significant aggregation of nests of size S_1 for radii greater than 3 m, and a significant aggregation of nests of size S_1 around nests of size S_2 for radii greater than above 6 m (Fig 5.2A). Nests of size S_2 do not display a significant aggregation at any scale. For both nest sizes, there was an indication (negative values of the L function at small *r*) of a non-significant small-scale repulsion between nests, which was most pronounced for nests of size S_2 in relation to other nests of size S_2 (Fig 5.2A). In 2010, there was no significant aggregation. Aggregation of nests of size S_1 and S_2 with radii larger than 3 and 2 m, respectively, as visually suggested by Fig 5.2A and Fig 5.2B,

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was not significant. Nests of size S_1 were more regular distributed from nests of size 2 at all scales as indicated by negative values of the L function at all *r*. There seems to be strong short distance inhibitory responses, which are most pronounced for nests of size S_2, and between nests of size S_2 and S_1 (Fig 5.2B).

Next, the fitted inhomogeneous Poisson model, using the non-parametric density estimate, was taken as the reference upon which the model for inter-point interactions was build. In 2010, the inhomogeneous Poisson model did not properly describe the observed patterns of size S_1 and size S_2 nests (Fig 5.3A, B).
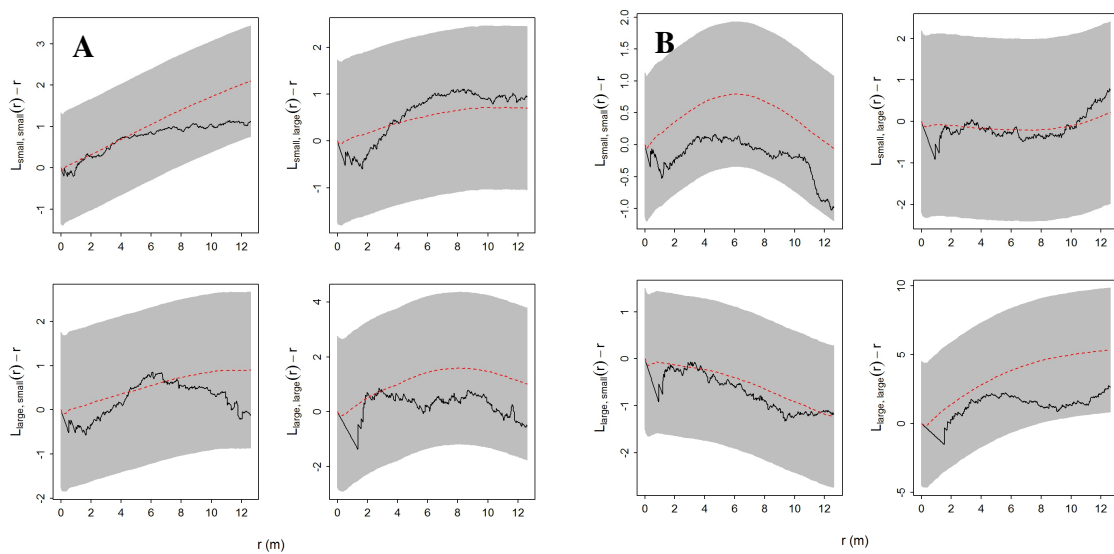
Inhomogenous Poisson model

Fig 5.3 Estimated L-function for each pair of nest sizes, as a function of *r* in 2009 (A) and 2010 (B); within plots A and B, the left top pane shows the spatial distribution of nests size class S_1, the right top pane the arrangement of nests of size class S_1 around class S_2, the left bottom pane the arrangement of nests of size class S_2 around class S_1, and the right bottom pane the spatial spatial distribution of nest size class S_2. The red dashed line represents the expected value of the L-function under the inhomogeneous Poisson process, while the grey band represents the simultaneous 95 % critical envelopes.

In 2009 and 2010, the L function, taking into account the estimated trend for each nest size class, improved the fit as compared to the expected L function, but there were still deviations, specifically at short distances (Fig 5.3A, B). These indicated short-range nest interactions.
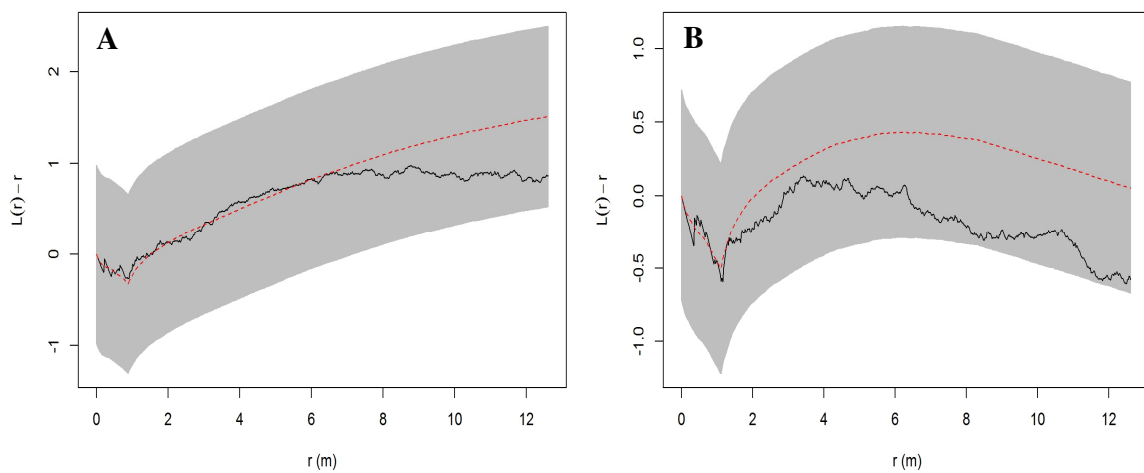
Common Inhomogeneous Strauss model

Fig 5.4. Estimated inhomogeneous Strauss model common to all nest sizes as a function of *r* in 2009 (A) and 2010 (B); the red dashed line represents the expected value of the L-function under the inhomogeneous Poisson process, while the grey band represents the simultaneous 95 % critical envelopes.

In 2009, the common inhomogeneous Strauss process further improved the fit (Fig 5.4A); there was some repulsion between nests, with an estimated interaction distance $r = 0.889$ m and a fitted interaction parameter $\hat{\gamma} = 0.306$ (moderately strong repulsion among nests). The fit of the Strauss model was better than that of the inhomogeneous Poisson process ($\Delta\log(pLik) = 26.10125$) and significant ($p \leq 0.004$; 249 simulations of the null

pattern). In 2010, the global inhomogeneous Strauss model improved the fit at short distances for the whole pattern, regardless of the nest size (Fig 5.4B). Similar to 2009, there is some repulsion between nests, with an estimated interaction radius $ir = 1.11$ m and a fitted interaction parameter $\hat{\gamma} = 0.213$, which is stronger repulsion than in 2009. The fit of the Strauss model is better than the inhomogeneous Poisson process ($\Delta\log(pLik) = 33.011$) and significant ($p \leq 0.004$, 249 simulations of the null pattern).
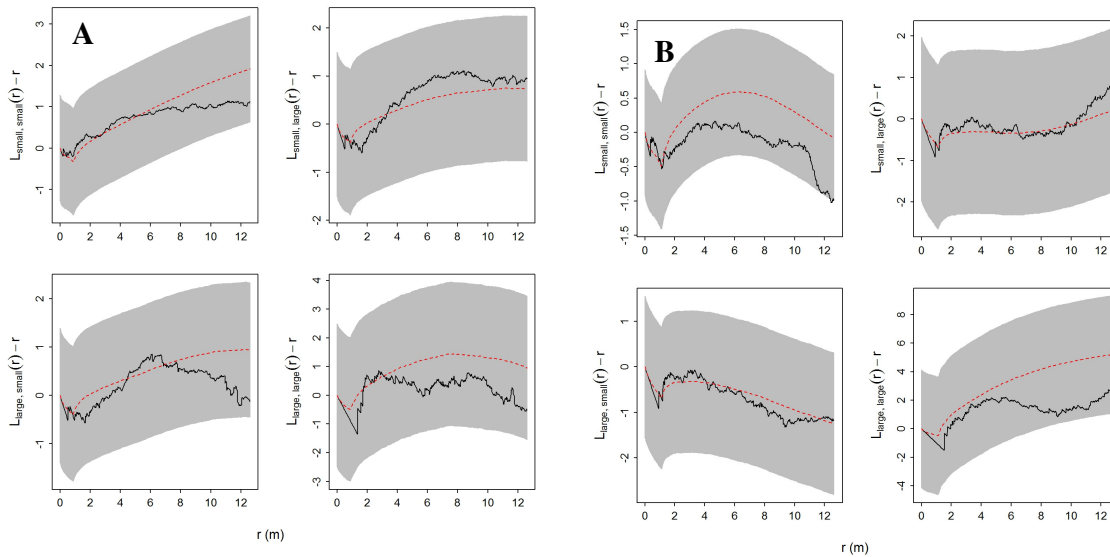

Fig 5.5 Estimated common inhomogeneous Strauss model for each pair of considered nest sizes as a function of r in 2009 (A) and 2010 (B); within plots A and B, the left top pane shows the spatial distribution of nests size class S_1, the right top pane the arrangement of nests of size class S_1 around class S_2, the left bottom pane the arrangement of nests of size class S_2 around class S_1, and the right bottom pane the spatial spatial distribution of nest size class S_2. The red dashed line represents the expected value of the L-function under the common inhomogeneous Strauss process, while the grey band represents the simultaneous 95 % critical envelopes.

It seems that nests of size S_2 have greater repulsion distances compared to nests of size S_1. However, a common Strauss process is inappropriate at short distances, for all nest sizes in both years (2009 and 2010) the fit of the spatial structure of size S_1 nests was proper, but not that of size S_2 or of reciprocal spatial relations (Fig 5 A, B). The common Strauss process was used in order to explain inter-nest repulsive interactions that do not depend on nest size. The fit was significant and better than that of the inhomogeneous Poisson process (Table 5.1). To test the repulsion between nests per each nest size class another model-inhomogeneous Strauss process per nest size was used (Fig 5.5).

Multi-type Strauss model
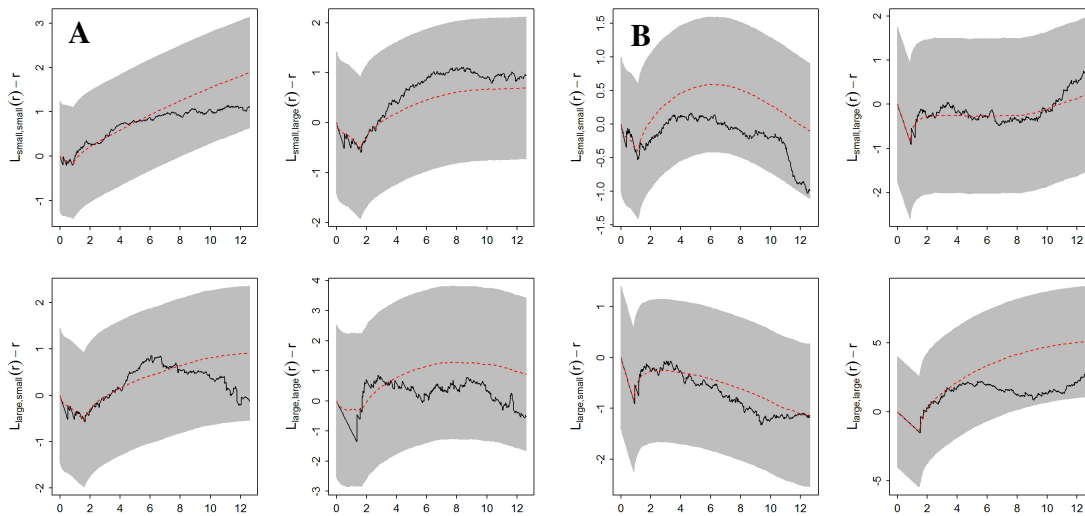
Fig 5.6. Estimated multi-type Strauss model for each pair of considered nest sizes as a function of *r* in 2009 (A) and 2010 (B); within plots A and B, the left top pane shows the spatial distribution of nests size class S_1, the right top pane the arrangement of nests of size class S_1 around class S_2, the left bottom pane the arrangement of nests of size class S_2 around class S_1, and the right bottom pane the spatial spatial distribution of nest size class S_2. The red dashed line represents the expected value of the L-function under the inhomogeneous multi-type Strauss model, while the grey band represents the simultaneous 95 % critical envelopes.

In 2009, the multi-type Strauss inhomogeneous model (Fig 5.6A) improved the fit over the Strauss model. The distances over which interactions occurred were estimated at $ir_{11} = 0.89$ m, $ir_{12} = ir_{21} = 1.59$ m and $ir_{22} = 1.69$ m. The fitted interaction parameters were $\hat{\gamma}_{11} = 0.490$, $\hat{\gamma}_{12} = 0.367$ and $\hat{\gamma}_{22} = 0.429$ (moderately strong repulsion among nests). The improvement over the null model was larger than that of the Strauss model ($\Delta\log(pLik) = 34.09144$) and also significant (p ≤ 0.004, 249 simulations of the null pattern). However, there was an important departure at short distances for the spatial structure of nests of size S_2, which seemed to be more under-dispersed than indicated by the multi-type Strauss model.

In 2010, this model (Fig 5.6B) improved the fit of the Strauss model. The interaction radii are $ir_{11} = 1.15$, $ir_{22} = 1.45$ and $ir_{12} = 0.85$. The fitted interaction parameters were $\hat{\gamma}_{11} = 0.303$, $\hat{\gamma}_{12} = 0.0$ and $\hat{\gamma}_{22} = 0.0$; these last two interaction parameters indicated complete repulsion between nests of size 2 and between S_1 and size S_2 up to the interaction radii. The improvement over the null model was larger than that of the Strauss model ($\Delta\log(pLik) = 42.590$) and significant (p ≤ 0.004, 249 simulations of the null pattern). There were some departures between the estimated L function and the expectation from the model, especially for nest of S_1 and S_2, not on short distances. However, for radii larger than 2 m, the non-parametric trend did not seem to be appropriate. Because of these reasons the Multi-type hard-core Strauss process had to be applied. However, Multi-type Strauss process (Fig 5.6) explained the interactions depending on the nest size, significantly and better than previous models. However, the distribution of nests was always more aggregated for younger nests and more regular for older nests (Table 5.2).

Table 5.2 Estimated nest distribution for two nest age classes (young and old) in the experimental plot (50 m x 150 m).

	2009		2010	
Nest age	Nest distribution trend	Nest age	Nest distribution trend	
young-young	clumped-regular	young-young	regular	
young-old	clumped	young-old	regular-clumped	
old-young	clumped-regular	old-young	regular	
old-old	regular	old-old	regular	

Multitype/hard core Strauss model
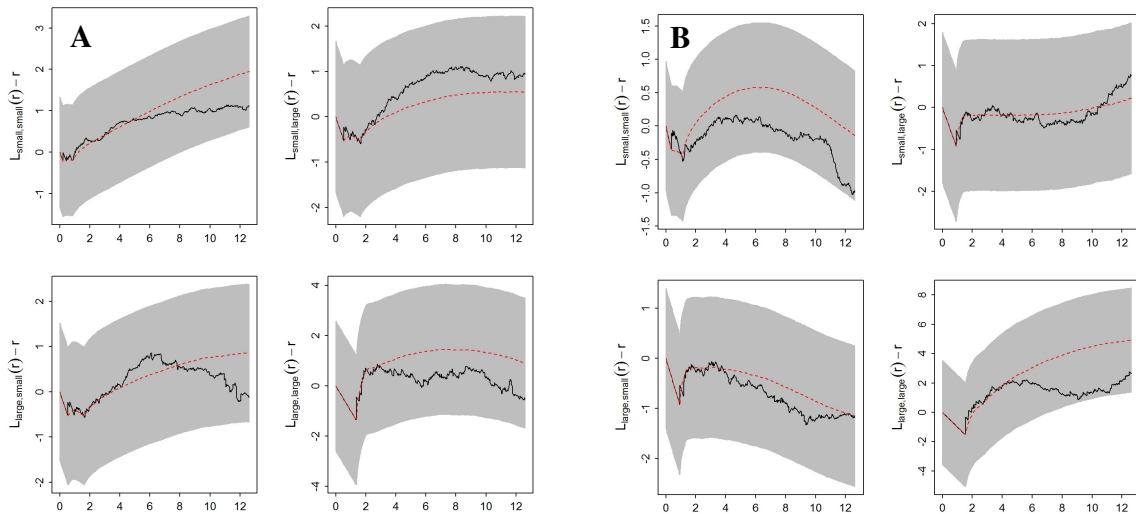
Fig 5.7 Estimated multi-type/hard core Strauss for each pair of considered nest sizes as a function of *r* in 2009 (A) and 2010 (B); within plots A and B, the left top pane shows the spatial distribution of nests size class S_1, the right top pane the arrangement of nests of size class S_1 around class S_2, the left bottom pane the arrangement of nests of size class S_2 around class S_1, and the right bottom pane the spatial spatial distribution of nest size class S_2. The red dashed line represents the expected value of the L-function under the inhomogeneous multi-type/hard-core Strauss model, while the grey band represents the simultaneous 95 % critical envelopes.

In 2009, the fit of the multi-type/hard core Strauss process (Fig 5.7A) indicated interaction radii ir_{11} = 0.85 m, ir_{12} = 1.63 m and ir_{22} = 2.02 m and their respective associated interaction parameters $\hat{\gamma}_{11}$ = 0.449, $\hat{\gamma}_{12}$ = 0.425 and $\hat{\gamma}_{22}$ = 2.130. Hard-core radii were hr_{11} = 0.221 m, hr_{12} = 0.538 m and hr_{22} = 1.376 m (Table 5.3) The estimate for the interaction parameter $\hat{\gamma}_{22}$ was higher than its theoretical upper bound (1), which would mean no interaction between nests, indicating that the model was ill defined. There was some aggregation of nests of S_2 at short distances that the density trend was not able to account for. The improvement over the null model was the best of the tested models (Δlog($pLik$) = 62.060) and also significant (p ≤ 0.004, 249 simulations of the null pattern).

In 2010, the fit of multi-type hard core Strauss model (Fig 5.7B) indicated interaction radii ir_{11} = 1.12, ir_{12} = 1.38 and ir_{22}= 2.81 and their respective associated interaction parameters $\hat{\gamma}_{11}$ = 0.286, $\hat{\gamma}_{12}$= 1.400 and $\hat{\gamma}_{22}$ = 0.757. Estimates of hard-core radii were hr_{11} = 0.368, hr_{12} = 0.934 and hr_{22} = 1.53. The improvement over the null model was the best of the tested models (Δlog($pLik$) = 51.559) and also significant (p ≤ 0.004, 249 simulations of the null pattern). This model (Fig 5.7) included the interaction radius within which the probability of finding further nests was reduced and the interaction parameters were higher than estimated from the previous Strauss model. It was slight improvement; however it is not known whether it was significant.

Table 5.3. Estimated hard-core and interaction radii (m) between nest age classes (young and old) in the experimental plot (50 m x 150 m).

	2009			2010	
Nest age	Hard-core radii (m)	Interaction radii (m)	Nest age	Hard-core radii (m)	Interaction radii (m)
young-young	0.2	0.9	young-young	0.4	1.1
young-old	0.5	1.6	young-old	0.9	1.4
old-old	1.4	2.0	old-old	1.5	2.8

Discussion

It was predicted that the distribution of nests would be more regular 1) for older than for young colonies and 2) at high than at low nest densities. These predicted hypotheses were confirmed for the harvester ant *M. barbarus* in NE Spain. The implicit assumption here is that nest size is related to colony age; larger nests represent 'older' colonies.

Exclusion zone. The multi-type Strauss hard-core model provided an estimate of the zone around a nest entrance from which other nests were completely excluded. The zone was smaller for younger nests (0.2 m and 0.4 m in 2009 and 2010, respectively) and larger for older nests (1.4 to 1.5 m in 2009 and 2010, respectively). Ants likely distinguished this zone from the rest based on specific, odorous markers-pheromones (Cammaerts and Cammaerts 2001). They perform nest-area marking laying down colony specific compounds. Ants can place large quantities of pheromones in the vicinity of a nest in order to define their own area and often to distinguish nest-mates from alien

workers (Grasso et al. 2005). Beyond of the exclusion zone, the level of aggression against other colonies decreases (Knaden and Wehner 2003).

Small-scale distribution patterns. At a small scale (1-13 m), the distribution of older nests was more regularly than that of the young nests. The minimal distance where the queen establishments occurred, estimated by the interaction distance, was 0.9 m in 2009 and 1.1 m in 2010 for young nests, and 2.0 m in 2009 and 2.8 m in 2010 for old nests. It is likely that the area close to nests was more intensively used for foraging and therefore better defended. The larger interaction distances when old nests were involved were probably caused by a stronger defense response of older colonies, which have more workers (Diaz 1991). It is known for other ant species that the defense decreases with increasing distances from the nest entrance (Knaden and Wehner 2003).

In 2009, the distribution of young nests varied from more aggregated at a scale of 2-5 m to more regular at longer distances. Young nests tended to be more regulated at scale of 0.5 to 4 m around old nests while the trend was more aggregated at scale of 4-12 m. In 2010, young nests were more regularly distributed. The fact that the distribution of young nests changed from 2009 to 2010 can be explained by a lower nest density in 2010. We do not know why nest density decreased from 2009 to 2010. It is possible that resources in 2010 were more limited, although there are no indications for that large-scale distribution patterns. Large scale trends in nest distribution (13-50 m) were removed from the data set prior to analysis and were, therefore, not part of this study. However, it is clear those large-scale factors, such as soil characteristics (Enzmann and Nonacs 2010), orientation of slope (Azcárate et al. 2007), land use and field management (Diaz 1991; Baraibar et al. 2009), topography (Crist and Wiens 1996) or microclimate (Kilpeläinen et

al. 2008) may have influenced nest distribution. Here, no information was included about the slope orientations or microclimate. The soil characteristics, topography and agricultural management except of tillage seems are not correlated to the nest distribution in this area. Baraibar et al (2011) did not find the correlation between the soil characteristics, topography and agricultural management except of tillage to the nest density. The increasing of nest density leads to more regular spatial distribution and therefore to higher predation rates. However, the future study in this area may find the influence of these characteristics.

It is clear from the results of this study that weed seeds have a low chance to escape predation, because 1) nest density was really high and 2) nests tended to be regularly distributed, providing little opportunity for seeds to escape discovery by ants. Therefore, seed predation by harvester ants seems to be a powerful tool against weeds in dryland cereal fields.

Because overdispersion decreases with increasing nest density, it is important to keep nest densities high; this is the best way to ensure a field-wide coverage by harvester ants. Shallow tillage or minimum tillage may facilitate the establishment of the founding queens, especially after multiple years of no tillage (Baraibar et al. 2011b). This could lead to the increase of ant nest densities and to regular distribution trends, decreasing the probability of having field areas with few or no nests.

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CHAPTER 6

General discussion

General discussion

The temporal and spatial variability in seed predation in dryland cereal fields was investigated. This study confirmed that seed predation by harvester ants in dryland cereals was high (Baraibar et al. 2009). Moreover, rates were among the highest ever recorded on arable fields (46-100%). This study showed that the timing of weed seed shed overlapped with the period of highest demand. This overlap caused that most seeds that were shed during the season were collected by harvester ants, and prevented them from entering the soil bank, thus limiting weed problems in subsequent season´s. However, a small proportion was not consumed because they escaped or avoided seed predation through crop harvest or low preference. The timing of crop harvest was one of the important factors for weeds 1) that shed seeds prior to harvest and 2) that shed seeds during the harvest. First, the delay or advance of the harvest determined the proportion of seeds that could be shed at harvest. More time available for seeds to be exposed to the predators allowed higher seed losses, it was the case of the harvest delay. The advanced harvest induced seed shed of most of the seeds at harvest. Second, certain weeds have the phenology similar to the crop and therefore they escape predation after being shed at harvest and then covered with the straw. Those seeds became hidden under the straw and inaccessible to ants. In addition, the straw was always removed by farmers in 4–5 weeks. Some seed species, such as *Bromus diandrus* L.*,* were shed before harvest but escaped predation by being less preferred by ants. Seed size played an important role in avoiding predation, because small seeds, such as *Papaver rhoeas* L., were buried faster and therefore available to ants for a shorter period of time than large seeds. Of the seeds of *B. diandrus* about 15 to 25% avoided predation because of low preference to ants, while 0–

29 % escaped through crop harvest. Of the seeds of *P. rhoeas,* 20–32% escaped through crop harvest, while another 13–17% escaped by fast burial into the soil. It is clear that the weeds *B. diandrus*, *L. rigidum* and *P. rhoeas* would cause more problems in arable fields without presence of seed predators.

In this study, it was shown that harvester ants will prevent weed seeds to enter into the subsoil and to germinate in the subsequent season. This knowledge could help to the farmers in order to improve current weed control by herbicides. However, a small proportion was not consumed because they escaped or avoided seed predation due 1) the variability over the season or 2) the variability in space over the field. Weed seeds were able to escape predation. First, the timing of harvest was one of the important factors because some weed species shed seeds prior to harvest and other after harvest. The decision of the farmers to delay or to advance harvest will increase or decrease opportunity to these seeds to escape predation. After harvest, the straw that covered the seeds is always removed by farmers in 4–5 weeks. The most of seeds shed during the harvest became temporally or completely inaccessible to harvester ants. Removing the straw bit earlier should allow the availability of seeds. Seed predation after harvest is low (Baraibar et al. 2009) but still exists. The weed populations that were not favorable for ants or with small seeds cannot be fully regulated by ants.

Little is known about how spatial variability in seed predation rates may influence overall total seed predation rate or opportunities to weeds to persist. The spatial distribution of ant nests might influence seed predation rates due to unequal foraging intensities within a field. In areas where nests occur more clumped, weed seeds may escape predation because there may be locations that are less frequently visited, where

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ants are less abundant or where they never forage. The spatial nest arrangement appeared to be non-random. At medium scale, large nests were more or less regularly distributed, but small nests were not. Especially larger nests are known to be more aggressive to members of other colonies or other species (Diaz 1991). The clumped distribution of small nests can be understood by the process of new queen establishment and competition with larger nests. The nest density decreased from 2009 to 2010. It is possible that it could be due the less availability of resources but it is not confirmed. The clumped distribution trend decreases with increasing density of colonies, here it was observe an over-dispersion trend of small nests in 2009. The large scale trends were removed from the data-set because the spatial pattern of the nests was geo-referenced and analyzed at the field scale. The spatial distribution trends at this scale may be affected by landscape, topography, microclimate, vegetation land use, level of disturbance and field management, altitude, elevation, etc.

A regular and high occupancy of the field by ant nests could ensure successfully seed predation throughout the field. According to Díaz (1992) and Baraibar et al. (2011), nest density around 1200 nests ha^{-1} with regular distribution would be desirable in arable fields. The regular spatial arrangement increases with higher nest density and decrease a chance to a seed to escape predation. Here, nest densities were approximate at or above the ideal density. Whatever the distribution of nests and ants, weed seeds had little opportunity to escape predation because predation rates were very high everywhere in the field.

Seed predation rate could be related to the spatial distribution of weed species. Weeds grow in patches. Furthermore, seed density may differ between patches, which

could affect seed predation rate. Therefore, the effect of the patch quality, defined as seed density in the patch, on seed predation by harvester ants in dryland cereals was investigated. It was expected that ants would respond directly density dependent to seed density. However, harvester ants responded with extremely high predation rates (99-100 %) to all densities of *Lolium multiflorum* L. seeds applied (1000-20000 seeds m^{-2}). Consequently, the response was density independent. Apparently, nest density in the field was so high (>300 nests ha^{-1}) that allowed the highest predation rates (99-100%). The high ant nest density may be related to the long period that the field had been managed without tillage (15 years). Tillage is known to influence nest abundance (Baraibar et al. 2009). Had a less-preferred species, such as *B. diandrus* been used, maybe the harvester ants would have removed fewer seeds and the response may have been direct density or inversely density dependent. Unfortunately, this study did not show to which minimal seed density ants could respond to. Applied densities ranged from 1000-20000 seeds m^2 and it is likely that all of them were perceived as a profitable resource for ants. It is still unknown whether ants respond to densities below 1000 seeds m^{-2}, i.e. from 10 to 100 seeds m^{-2}. In this range, predation rates could increase with higher densities (direct density-dependence) either decrease (inversely density dependence). In addition, harvester ants could show different responses to different weed species. Finally, the encounter rate was independent of the density. Not found patches occurred in areas with few nests.

Of the two components of seed predation, namely patch encounter rate and patch exploitation rate, there was only variability in the first and not in the second. Eight patches were simply not found; all patches that were found were completely depleted. In

certain areas within the field where nest density was lower than average, ants failed to encounter some patches or patches were encountered later. The delay in encounter could give seeds enough time to escape to the subsoil, after which they are inaccessible to ants (Westerman et al. 2012) Therefore, weeds could persist in areas with a low nest density.

Patch size could influence predation rates by harvester ants. Therefore, the patch size was varied from 0.25 to 9 m^2 and seeded with 2000 seeds m^{-2}. Estimated seed predation rate was highest in the largest patches (99-100%), and lowest in the smallest patches (78-94%). Ants appeared to be unable to find seven of small-sized patches. Larger patch size facilitated patch encounter rate due to larger perimeters and higher area-to-perimeter ratios (A/P). There are two reasons for that. Firstly, the higher patch perimeter increased the probability large that patches were encountered by scouts. Secondly, larger patches contained higher amounts of seeds, which constitute more profitable resources (López et al. 1993; Detrain et al. 2000). When patches were found, the exploitation rate was the same, regardless of the size. Seeds may escape seed predation if they occur in small patches, which may not be detected by harvester ants. Consequently, harvester ants could specifically reduce the density of seeds in larger patches. This is an advantage because larger patches cause more crop yield losses.

This research quantified temporal and spatial variability in seed predation in dryland cereals to investigate how this variability might influence the efficacy of granivory and provide opportunities to weeds to escape predation.

Despite temporal overlap in seed shed and predation rates for most weed species, a small proportion of the seeds of some other species (i.e. *P. rhoeas*, *B. diandrus*) were

not predated. For weeds that shed seeds relatively early, the timing of harvest will determine the duration of exposure of these seeds on the soil surface; during harvest, seeds will be hidden under the straw and no longer available to predators. However, most weeds follow the phenology of the crop, which means shedding seeds at harvest. These will largely escape predation. If farmers want to make optimal use of seed predation, they could decide to delay harvest to allow more seeds to be shed and predated by ants. Alternatively, farmers could remove the straw immediately after harvest to make seeds better accessible.

Seed predation also varied spatially. Three components were investigated, namely spatial nest distribution, seed patch size and patch quality (seed density).When nests are more regularly distributed over a field there are less constraints for ants to find and exploit all weed patches. The uniformity of nests increases with nests density therefore more workers will be available to encounter the patch. Regardless of their density or size, all patches will be completely depleted. However, the spatial arrangement of ant nests tended to be more or less over-dispersed, meaning that is some areas of a field hosted few or no nests. Therefore, some patches will not be found, in particular if they are small. This smallest patch size in this study corresponds to the seed shadow of a single plant. Because of the higher survival probability of those patches, they will grow, which would enhance the encounter rate with harvester ants in subsequent years. Therefore, the probability that harvester ants will find and predate weed patches is very high.

It is likely that constraints for the establishment of colonies like soil characteristics (Wiernasz and Cole 1995; Crist 1998; Enzmann and Nonacs 2010) may explain why some areas of the field have fewer nests, however, that was not tested in this

study. Shallow tillage or minimum tillage may facilitate the establishment of the founding queens, especially after multiple years of no tillage (Baraibar et al. 2011). The best period for tillage would be in September prior to nuptial flights and queen establishment because it may facilitate ants to tunnel the soil and construct chambers (Wiernasz and Cole 1995; Boulton et al. 2005; Enzmann and Nonacs 2010).

This study confirmed that harvester ants play an important role in control of the main weed species in dryland cereals. In order to decrease or remove constraints for this natural service, measures to improve conditions for nest establishments in the areas with a low presence of harvester ants could be considered. This can be beneficial because a higher nest density leads to higher predation rates and a more regular nest distribution.

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Conclusions

The main conclusions from this research are:

- regarding variability of seed predation in time:
  - ✓ Seed predation rates by harvester ants in dryland cereals were among the highest ever recorded on arable fields (46-100%). The reason for the high rates is that the timing of weed seed shed overlapped with the period of highest demand.
  - ✓ A small proportion of newly shed seeds were not consumed because of crop harvest, low preference or due the small seed size that allowed fast seed burial.

- regarding variability of seed predation in space:
  - ✓ The spatial nest arrangement appeared to be non-random. The large nests were more or less regularly distributed, but small nests tended to be more clumped.
  - ✓ The clumped distribution trend decreased with increasing density of colonies. The regular trends in nest distribution increased with nest density and decreased opportunity to weed seeds to escape predation due the higher predation rates in the field.
  - ✓ The encounter rate was independent of the seed density in a patch. Harvester ants responded with extremely high predation rates (99-100 %) to all densities of *Lolium multiflorum* seeds applied. The most likely explanation is that nest density in the field was very high (>300 nests ha^{-1}). The few patches that were not found occurred in areas with a low nest density.
  - ✓ Patch size influenced predation rates by harvester ants. Estimated seed predation rate was highest in the largest patches (99-100%), and lowest in the smallest

patches (78-94 %). This was caused by a lower encounter rate of small patches. Larger patch size facilitated patch encounter rate due to larger perimeters and higher area-to-perimeter ratios (A/P). When patches were found, the exploitation rate was the same, regardless of the size.

Conclusiones

Las principales conclusiones de este estudio son:

- referente a la variabilidad en el tiempo de la depredación de semillas:
  - ✓ La depredación de semillas por hormigas granívoras en cereales de secano fue de las más elevadas jamás descritas (46-100%). La razón es la coincidencia en el tiempo de la producción de semillas con el período de máxima actividad de las hormigas.
  - ✓ Una pequeña proporción de las nuevas semillas producidas no fueron consumidas debido a la cosecha del cereal, baja preferencia o a su pequeño tamaño que permite un rápido enterramiento en el suelo.

- referente a la variabilidad de la depredación de semillas en el espacio:
  - ✓ La distribución espacial de los nidos de hormigas no era aleatoria. Los nidos grandes estaban más regularmente distribuidos y los nidos pequeños más agregados.
  - ✓ La distribución agregada de los nidos tendió a decrecer con densidades crecientes. La tendencia regular incrementó con densidades de nidos más altas, disminuyendo la oportunidad de las semillas de malas hierbas de escapar a su consumo debido a elevados niveles de depredación en el campo.
  - ✓ La tasa de encuentro de los rodales fue independiente de las densidades de semillas presentes. Las hormigas granívoras respondieron con niveles de depredación extremadamente altos (99-100 %) a todas las densidades de semillas de *Lolium multiflorum* aplicadas. La explicación más plausible es la elevada densidad de nidos

en el campo (>300 nidos ha^{-1}). Los pocos rodales no encontrados y no consumidos estaban en zonas con baja densidad de nidos.

✓ El tamaño de los rodales influenció la tasa de depredación de semillas por hormigas granívoras. Los niveles de depredación estimados más elevados fueron para los rodales más grandes (99-100%), mientras que los más bajos fueron para los rodales más pequeños (78-94 %). Esto fue debido a la menor tasa de encuentro de los rodales pequeños. Los rodales de tamaño más grande fueron más fácilmente encontrados debido a perímetros más largos y una relación área-perímetro más alta. Cuando un rodal era encontrado, el nivel de consumo era el mismo, independientemente de su tamaño.

Conclusions

Les principals conclusions d'aquest estudi són:

- referent a la variabilitat en el temps de la depredació de llavors:
  - ✓ La depredació de llavors per formigues granívores en cereals de secà va ser de les més elevades mai descrites (46-100%). La raó és la coincidència en el temps de la producció de llavors amb el període de màxima activitat de les formigues.
  - ✓ Una petita proporció de les noves llavors produïdes no van ser consumides degut a la collita del cereal, baixa preferència o a la petita mida que permet un ràpid enterrament en el sòl.

- referent a la variabilitat de la depredació de llavors en l'espai:
  - ✓ La distribució espacial dels nius de formigues no era aleatòria. Els nius grans estaven més regularment distribuïts i els nius petits més agregats.
  - ✓ La distribució agregada dels nius va tendir a disminuir amb densitats creixents. La tendència regular va incrementar amb densitats de nius més altes, disminuint l'oportunitat de les llavors de males herbes d'escapar al seu consumo degut a elevats nivells de depredació en el camp.
  - ✓ La taxa d'encontre dels rodals va ser independent de les densitats de llavors presents. Les formigues granívores van respondre amb nivells de depredació extremadament alts (99-100 %) a totes les densitats de llavors de *Lolium multiflorum* aplicades. L'explicació més plausible és l'elevada densitat de nius en el camp (>300 nius ha^{-1}). Els pocs rodals no trobats i no consumits estaven en zones amb baixa densitat de nius.

✓ La mida dels rodals va afectar la taxa de depredació de llavors per formigues granívores. Els nivells de depredació estimats més elevats van ser per als rodals més grans (99-100%), mentre que els més baixos van ser per als rodals més petits (78-94%). Això fou degut a la menor taxa d'encontre dels rodals petits. Els rodals de mida més gran van ser més fàcilment trobats degut a perímetres més llargs i una relació àrea/perímetre més alta. Quan un rodal era trobat, el nivell de consum era el mateix, independentment de la seva mida.